“A Mario, mamma, papa’, Ale, Elis e Bea.
Le persone piu’ importanti della mia vita.”

UNIVERSITA' DI PADOVA  **FACOLTA' DI INGEGNERIA**

Algorithms for the automatic tracking of the blood vessels network in retinal images acquired by RetCam in newborns.

Laureanda: Eleonora Grespan

Relatore: Alfredo Ruggeri

Correlatore: Diego Fiorin

Corso di laurea specialistica in Bioingegneria

13 Luglio 2010 A.A. 2009/2010

Table of Contents

INTRODUCTION

Retinopathy of prematurity (ROP) is a disease that can be present in premature infants and which, if not correctly and promptly healed, can lead to retinal detachment and visual loss.

It is characterized by an abnormal development of retinal vasculature which doesn't reach the periphery of the retina. In fact, generally, the development of retinal network becomes complete around full term at 9 months.

Consequently preterm birth can carry many complications because the retina is not fully vascularized and the progression of this disease can easily bring to serious damage to the patient eyes. Recently, several advances have been done on the understanding and management of this pathology but ROP continues to be a leading cause of childhood blindness throughout the world.

ROP development has been classified in 5 stages, among which the so-called plus disease condition is a very critic one because it is the stage in which the eventuality of a surgery intervention has to be seriously considered. This stage is characterized by increased vascular dilation and abnormal changes of other parameters such as tortuosity.

These vascular changes are usually only qualitatively assessed by visual inspection of retinal blood vessels. Ophtalmologists and experts have to review a large number of imagess so the evaluation of these parameters requires frequent, time consuming and subjective intervention by them.

A challenging aim to detect symptoms of ROP in an easier and more objective way is the realization of a suite of algorithms able to automatically detect the vascular network in the retina and, basing on that, calculate the main parameters (tortuosity and width).

Several researchers realized methods able to detect abnormalities of vessels by automatically measuring shape, size, and length or network of the retina. However many of these previous techniques can be applied successfully only on adult images. They fail on vessel detection on infant images which are more difficult to analyze because of their less visibility of blood vessels, their global noise and the different thickness of blood vessels.

The purpose of this work is the development of an automatic system to detect and to track the blood vessels network in an objective and sensitive way from retina images of premature infants.

This work is based on a set of 40 images acquired from preterm newborns. The RetCam used to collect the images (Clarity Medical System, California) has a 130° field of view and the fundus camera has a VGA sensor. The resolution is 640 X 480 pixels.

In the first chapter the main characteristics of ROP are exposed. It is described its causes, classification, diagnosis and treatment.

In the second chapter a brief state of the art is presented, explaining which are the main problems related to the kind of images we can obtain from premature infants, and which are the most challenging aims of this work.

In the third chapter the method developed and utilized in this work is thoroughly explained.

The fourth chapter is dedicated to the description of the results we obtained.

Conclusions, discussion and further aims are explained on chapter five.

CHAPTER 1: RETINOPATHY OF PREMATURITY (ROP)

ROP (retinopathy of prematurity) represents one of the leading causes of blindness in infants in developed and emergent countries. It is fundamental being able to detect ROP promptly in order to diagnose and treat it early, in several cases solving the problem or avoiding serious complications.

1.1 DEFINITION

Retinopathy of prematurity is characterized by an abnormal retina vascularization which can take place in premature newborns.

In these babies the growth of retinal blood vessels doesn't reach the peripheral area of the retina.

In fact, usually, blood vessels begin their development, starting from the optic disk, during the 15^{th} gestation week continuing their growth and ramification until the 9^{th} month of pregnancy.

Several studies showed that retinal blood vessels grow starting from cells called spindle cells which develop from the optic nerve through the ora serrata. Spindle cells reach the ora serrata at 29^{th} week of gestation, whereas the blood vessels develop later and they reach the retinal periphery only during the last weeks of gestation.

Consequently in premature newborns the blood vessels accretion didn't have the time to reach all retina's areas and this lead to several kind of complications.

Beyond that, a relevant condition that allow the migration and maturation process of the spindle cells is the hypoxic environment in the uterus (PaO2=25 mmHg). This hypoxic condition strongly changes after birth and the PaO2 reach values of 70mmHg or more negatively influencing the retinal blood vessels development. In fact the hyperoxigenated blood lead to formation of dangerous free radicals that decelerate the spindle cells maturation process [15].

Retinopathy of prematurity is a complex disease caused by several factors and circumstances so the exact pathogenesis isn't completely clear yet.

1.2 CAUSES AND RISK FACTORS

The two main important causes related to the development of this pathology are considered a low birth weight (less than 1500 grams) and a low gestation age (less than 32 weeks) [18]. Generally, the more premature is the baby, the deeper is the sickness

Retinal blood vessels begin to develop 3 months after conception and complete their development at the time of normal birth. If an infant is born very prematurely, eye development can be disrupted. The vessels may stop growing or grow abnormally from the retina into the normally clear gel that fills the back of the eye. The vessels are fragile and can leak, causing bleeding in the eye. Scar tissue may develop and pull the retina low from the inner surface of the eye. In severe cases, this can result in vision loss.

In the past, routine use of excess oxygen to treat premature babies stimulated abnormal vessel growth. Currently, oxygen can be easily and accurately monitored, so this problem is rare.

Today, the risk of developing ROP depends on the degree of prematurity. Generally, the smallest and sickest premature babies have the highest risk.

Typically all babies younger than 30 weeks gestation or weighing fewer than 1500 grams at birth are screened. Certain high-risk babies who are born after 30 weeks should also be screened.

In addition to prematurity and low weight, other risks factors may include [16]:

- Brief stop in breathing (apnea)
- Heart disease
- High carbon dioxide (CO_2) in the blood
- Infection
- Low blood acidity (pH)
- Low blood oxygen
- Respiratory distress

- Slow heart rate (bradycardia)
- Transfusions

Luckily, the rate of ROP in moderately premature infants has decreased dramatically with better care in the neonatal intensive care unit. However, this has led to high rates of survival of very premature infants who would have had little chance of survival in the past. Since these very premature infants are at the highest risk of developing ROP, the condition may actually becoming more common again.

1.3 CLASSIFICATION

The international ROP classification (ICROP), used in order to code correctly epidemiological data, avails of a specific pattern to classify lesions [8].
It has been realized the first time in 1984 and then revised in 1987 and 2005 and it has been very useful in order to collect more information about this disease and in order to improve the knowledge of its development.
ICROP classification is based on several essential observations to do to describe the retinopathy, as the location, the extension, the stage, the blood vessels conformation.

1.3.1 LOCALIZATION

Considering the localization, the ocular fundus has been subdivided in three concentric zones centered on the optic disk:

- Zone 1 is the posterior zone of the retina, defined as the circle with a radius extending from the optic nerve to double the distance to the macula. Zone 1 is the most centrally located and ROP develops in this area if the retina in this area is most underdeveloped. Disease in zone 1 is more severe compared with disease limited to zones 2 or 3.
- Zone 2 is an annulus with the inner border defined by zone 1 and the outer border defined by the radius that is the distance from the optic nerve to the nasal ora serrata.
- Zone 3 is the residual temporal crescent of the retina.

1.3.2 EXTENSION

Considering the extension, it is evaluated basing on the horary localization as if the top of the eye was 12 on the face of a clock, that is basing on the "number of hours" characterized by pathology signs. As the observer looks at each eye, the 3-o'clock position is to the right and nasal in the right eye and temporal in the left eye, and 9-o'clock position is to the left and temporal in the right eye and nasal in the left eye.

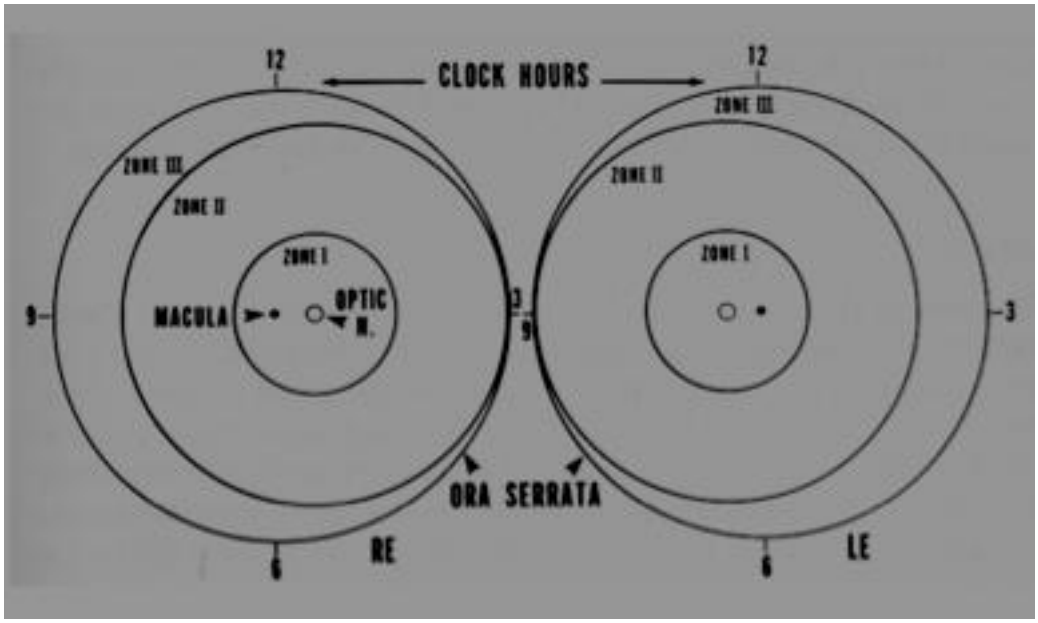

figure 1.1: localization and extension of ROP

1.3.3 PATHOLOGY'S STAGES

Prior to the development of ROP in the premature infant, vascularization of the retina is incomplete or immature. There are 5 stages that are usually used to describe the abnormal vascular response at the junction of the vascularized and avascular retina [16].

ROP STAGE 1: examining the premature newborns ocular fundus the image can show a peripheral area of the retina lacking of blood vessels, really well defined and bordered by an evident demarcation line from the rest of the retina, which is well vascularized. This line is a thin but definite structure that separates the avascular from the vascularized retina.

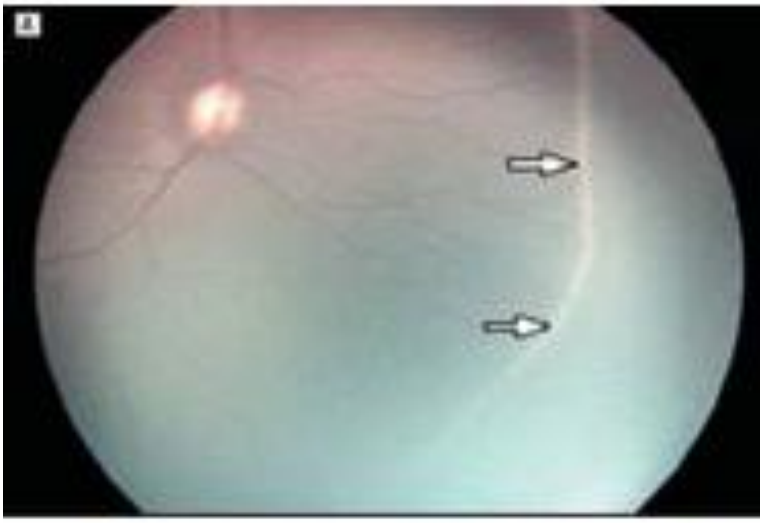

figure 1.2: ROP stage 1

ROP STAGE 2: the strong demarcation line that divides the two areas (one vascularized, the other without blood vessels) can disappear during the following weeks, and the blood vessels can develop in a normal way reaching also the peripheral area of the retina.

Nevertheless the pathology can also advance achieving the stage 2, in which the demarcation line assumes the morphology of a ridge. The ridge is the hallmark of stage 2 ROP. It arises in the region of the demarcation line but it has height and width and extends above the plane of the retina. The ridge may change from white to pink and vessels may leave the plane of retina posterior to the ridge to enter it.

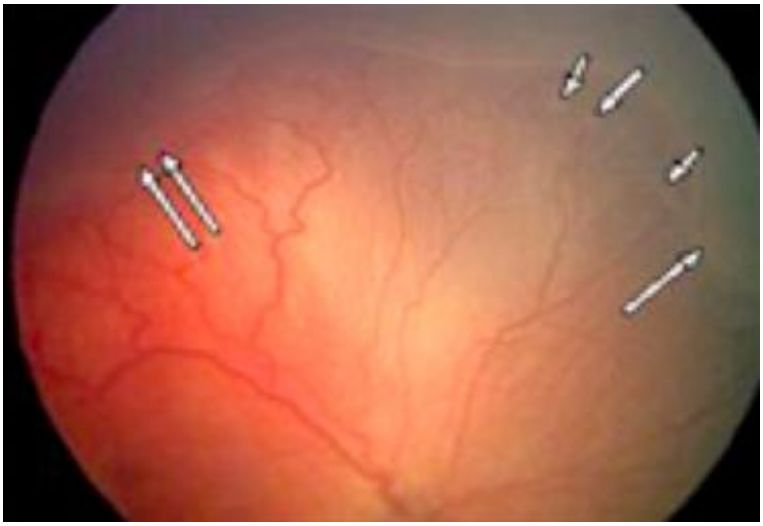

figure 1.3: ROP stage 2

ROP STAGE 3: from ROP stage 2 the pathology can regress spontaneously but it also can develop into an extraretinal blood vessels proliferation; in stage 3, extraretinal fibrovascular proliferation or neovascularization extends from the ridge into the vitreous. This extraretinal proliferating tissue is continuous with the posterior aspect of the ridge, causing a ragged appearance as the proliferation becomes more extensive.

The severity of a stage 3 lesion can be subdivided into mild, moderate, or severe depending on the extent of extraretinal fibrovascular tissue infiltrating the vitreous.

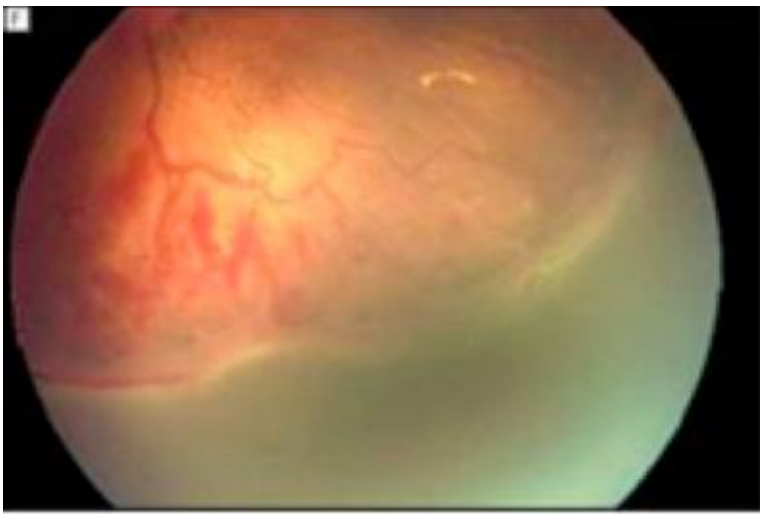

figure 1.4: ROP stage 3

ROP STAGE 3 PLUS : if the development of the disease is very fast and the posterior vessels are characterized by tortuosity and dilation associated to retinal hemorrhages, the pathology is classified as stage 3 plus which represents the point of no return for this kind of disease, the moment in which is indispensable to consider the possibility of a surgery intervention.
The ROP in stage 3 that requires treatment is usually called threshold disease.

figure 1.5: ROP stage 3 plus

ROP STAGE 4: it is divided into extrafoveal (stage 4A) and foveal (stage 4B) partial retinal detachments. Stage 4 retinal detachments are generally concave and most are circumferentially oriented. The extent of retinal detachments depends on the number of clock hours of fibrovascular traction and their degree of contraction.

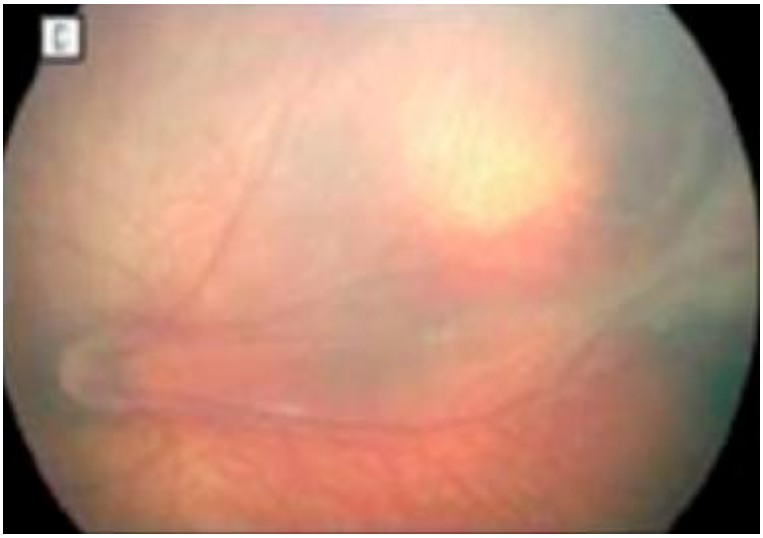

figure 1.6: ROP stage 4

ROP STAGE 5: it is characterized by a total retinal detachment. Retinal detachments are generally tractional and may occasionally be exudative.

figure 1.7: ROP stage 5

1.3.4 PLUS DISEASE

Along with the changes described earlier at the leading edge of the abnormally developing retinal vasculature, additional signs indicating the severity of active ROP may occur. These include increased venous dilatation and arteriolar tortuosity of the posterior retinal vessels and may later increase in severity to include iris vascular engorgement, poor papillary dilatation and

vitreous haze. This important constellation of signs in the original classification was referred to as plus disease. Subsequent multicentered clinical trials have used a “standard” photograph to define the minimum amount of vascular dilatation and tortuosity required to make the diagnosis of plus disease. This definition has been further refined in the later clinical trials in which the diagnosis of plus disease could be made if sufficient vascular dilatation and tortuosity are present in at least 2 quadrants of the eye. [8]
Plus-disease may be present at any stage.

1.3.5 PRE-PLUS DISEASE

Pre-plus disease is defined as vascular abnormalities of the posterior pole that are insufficient for the diagnosis of plus disease but that demonstrate more arterial tortuosity and more venous dilatation than normal. Over time, the vessel abnormalities of pre-plus may progress to plus disease as the vessels dilate and become more tortuous [8].

1.3.6 AGGRESSIVE POSTERIOR ROP

An uncommon, rapidly progressing, severe form of ROP is designated as AP-ROP. If untreated, it usually progresses to stage 5 ROP. The characteristic features of this type of ROP are its posterior location, prominence of plus disease, and the ill-defined nature of the retinopathy. Aggressive posterior ROP is observed most commonly in zone 1, but may also occur in posterior zone 2. Early in the development of AP-ROP, the posterior pole vessels show increased dilation and tortuosity in all 4 quadrants and it’s often difficult to distinguish between arterioles and venules because of the significant dilation and tortuosity of both vessel types [8].

1.3.7 ROP PHASES

From this classification it emerges that the disease develops through a progressive process characterized by stages of different severity. In some cases the disease can spontaneously evolve until a complete healing and a natural blood vessels’ maturation.

Patients at risk of ROP or affected by ROP are periodically controlled, following a protocol in order to localize the lesion and to monitor the evolution of the disease.

By a clinic point of view, ROP can also been subdivided in two phases:

- Acute phase;
- Cicatricial phase..

The main difference between the two forms is the presence of a vascular component. The development of the disease lead to the cicatricial phase which can lead to the retinal detachment.

The acute phase can be subdivided in the first 4 stages whereas the 5th stage is called the cicatricial phase.

1.4 SCREENING

High risk premature infants are usually monitored by a retinal specialist or pediatric ophtalmologist during their staying in a neonatal care unit. Guidelines for screening can lightly change among different hospitals but generally [15]:

- Infants born at 23-24 weeks should be examined within three to four weeks
- Infants born at or beyond 25 or 28 weeks should be examined by the fourth to the fifth week
- Infants born after 29 weeks should be examined prior to discharge from the hospital.

Nevertheless, all premature infants are at higher risk for eye and vision complications so every children born under 32 weeks or that weigh less than 1500 grams have to examined.

1.5 DIAGNOSIS

Despite of a big amount of studies about the effect of E vitamin or other antioxidants and a severe control in oxygen administration to preterm newborns, their positive effect is not so clear.

According with this situation, early diagnosis and screening assume a strategic role because they allow to control how the situation evolves and to decide the best moment for an intervention that, in most cases, can solve or strongly reduce the problem.

An effective protocol for ROP screening is based on two essential criterions:

- how to choose which newborns to control;
- how to choose how often to examine them.

The American Association for Pediatric Ophtalmology and Strabismus, the American Academy of Ophtalmology and the American Academic of Pediatric related in 1997 a document called “Joint Statement”, reviewed in 2001, in which is stated that every newborn who weighs less than 1500 grams and/or with gestational age less than 28 weeks have to be examinated and screened.

At the moment the diagnosis is made observing the retina, after pupillary dilatation using eye drops, by indirect ophtalmoscopy. Examination of the retina of a premature infant is performed to determine how far the retinal blood vessels have grown (the zone), and whether or not the vessels are growing flat along the wall of the eye (the stage). Retinal vascularization is judged to be complete when vessels extend to the ora serrata [18].

The exam can also be realized by digital ophtalmoscopy RetCam, a technology which uses lenses characterized by a wide field of view (130°-160°), that allows the analysis of both the posterior pole and the peripheral area of babies’ retina. In this way the images can be collected, archived and compared. The images can be interchanged among different centers using the web, evaluating and suggesting the best way to proceed according to each case.

The analysis of the retinal images let to understand to which stage belongs the disease. The stages classification is fundamental in order to decide the treatment.

An adverse prognostic element is the appearance of retinal vessels congestion and tortuosity

1.6 TREATMENT

Despite of the relevant progress about ROP pathogenesis, nowadays the only effective approach to stop or to reduce the development of the disease is surgical intervention

Some new approaches are based on administration of substances that control angiogenesis and blood vessels proliferation but these methods are still under study

Timing is one of the most important factors that make the treatment successful in ROP, because the disease can advance very quickly and delayed treatment often reduces the chance of success. The rapidly progressing ROP is called Rush Disease and it is usually associated with very extensive or aggressive growth of abnormal blood vessels. As explained above, abnormal dilatation of retinal veins with florid abnormal new vessels is called Plus Disease.

The treatment's goal is to destroy the retina that is deprived of retinal vessels. This helps to shrink the new vessels and prevents the formation of dense scars that usually follow. The dense scars cause traction on the retina. The result is distortion of the normal orientation of the retina and impairment of vision. In other cases, the retina detaches from the wall of the eye. The aim of the treatment is to extinguish the seeds of growing abnormal vessels in the eye, ultimately preventing the retina from detaching or from being severely distorted.

Currently the normal treatment in case of ROP in acute phase are [17]:

- Cryotherapy
- Laser Therapy
- Cryotherapy+laser therapy

CRYOTHERAPY: this technique has been used since the 1970 to treat ROP. By this method a regional retina destruction is done using a probe to freeze the desired areas. However, cryotherapy is no longer preferred for routine avascular retinal ablation in premature babies, due to the side effects of inflammation and swelling.

LASER THERAPY: using this technique the destruction of the avascular retina is preformed with a solid state laser photocoagulation device. Laser therapy has shown to be as effective as cryotherapy but with less systemic side effects.

In case of a severe stage of ROP (stage for or 5) the following treatments can be done:

- Scleral Buckling
- Vitrectomy

SCLERAL BUCKLING: this intervention can be necessary if a detachment of the retina occurs. It is a procedure that places a band around the globe of the eye This brings the retina back into contact with the inner layers of the eye.

VITRECTOMY: it is used to remove scar tissue from within the eye in the more severe cases of ROP. During the vitrectomy, the lens of the eye is usually removed.

Due to the difficulties of performing these surgeries on the small eyes of premature infants, these surgeries are usually performed only by a small number of ophtalmologists with extensive experience in ROP.

1.7 CONCLUSIONS

Due to the complexity of this pathology, making decisions about the way to proceed in order to limit the damage of ROP development but at the same time avoiding or limiting difficult interventions, it's a challenging task. The possibility of having an automatic device that provides useful information to the clinician about the actual state of the eye under exam would represent an important aid and would improve the quality of care of premature infants. The realization of a software that automatically detects the stage of ROP basing on RetCam images, would standardize and make more objective the way to proceed and would give more information to the ophtalmologists that could easier decide how to treat the pathology before that complex interventions as vitrectomy or scleral buckling were necessary.

In this work we developed a suite of algorithms that, basing on RetCam images, automatically track the retina blood vessels network. This represents

a fundamental step for the realization of a software able to calculate indicative parameters as tortuosity and dilation.

CHAPTER 2: RETCAM IMAGES

So far we described the main features of ROP (Retinopathy Of Prematurity), underlying who are the infants that have to be checked and the importance of an early detection of the disease and of a periodic screening to observe how the disease develop and to decide if a surgical intervention is necessary.

Premature newborns often present several kinds of pathophysiological problems and different complications. In most cases they manifest ROP in different stages and indirect ophtalmoscopy doesn't represent a reliable and effective method to correctly diagnose the disease because the significant area of the retina to examine is the most peripheral.

The use of images from RetCam allows a more accurate way to analyze and categorize the images from the experts who also can collect, register and interchange them.

The hallmark of ROP is abnormal retinal vasculature and the analysis of two important features leads to the decision by ophtalmologists about the initial treatment: dilatation and tortuosity.

Dilatation and tortuosity of the retinal vessels at the posterior pole in two or more quadrants, termed plus-disease, emerged as a sign of high risk pre-threshold ROP.

An objective and early detection of plus-disease represents a fundamental step in ROP care but presently it depends on the ophtalmologist's subjective assessment.

The aim of this project is to develop a suite of algorithms and implement them as prototype computer programs for the analysis of retinal images acquired in newborns with RetCam, for the tracking of vessels and the following estimation of vascular tortuosity and dilation, in order to classify the images as belonging to normal, Pre-Plus or Plus subjects.

2.1 RETCAM: ADVANTAGES

As described in the previous chapter, the hallmarks of ROP are usually located in the most peripheral area of the eye. In fact this is the zone in which retinal blood vessels grow during the last weeks of gestation.

In order to treat the baby in the best way it's fundamental an attentive monitoring of the retinal condition. The use of RetCam allows to obtain images of the eye that can be collected and compared leading to a more effective and reliable process of screening, diagnosis or follow-up of the patient.

The digital technology enables to obtain images that give information about the entire extension of the lesion, because of the wide RetCam field of view. In this way, the ophtalmologists are able to perform a more exhaustive and objective analysis of the patient condition [19]. The RetCam represents an essential device to early diagnosis and gives the possibility to share images or videos among specialized centers improving the quality of care.

RetCam technology also allows the realization of fluoroangiography, which can represent an important technique during the pre and post surgical follow-up.

2.2 RETCAM: DRAWBACKS

As explained above, RetCam represents a fundamental device in ROP care and enables to obtain more accurate and standardized diagnosis and decisions.

Nevertheless there still are several problems to consider and several aspects that should be improved to obtain decisions in a more subjective way, mainly considering that in this kind of disease timing in treatment represents one of the most important things and the surgical treatment of a newborn eye is an issue that has to be evaluated having as more information as possible.

Nowadays ophtalmologists have to take decisions basing on frequent, time consuming and logistically difficult interventions. Even the analysis of RetCam images is a time consuming and subjective issue. The

ophtalmologist has to evaluate a large number of images that often present some problems.

The main drawbacks of RetCam images (mainly if compared to fundus camera images of adult's eyes) are:

- Low contrast

figure 2.1: low contrast image

- Small dimension of blood vessels

figure 2.2: thin blood vessels

- Non-uniform illumination of the image

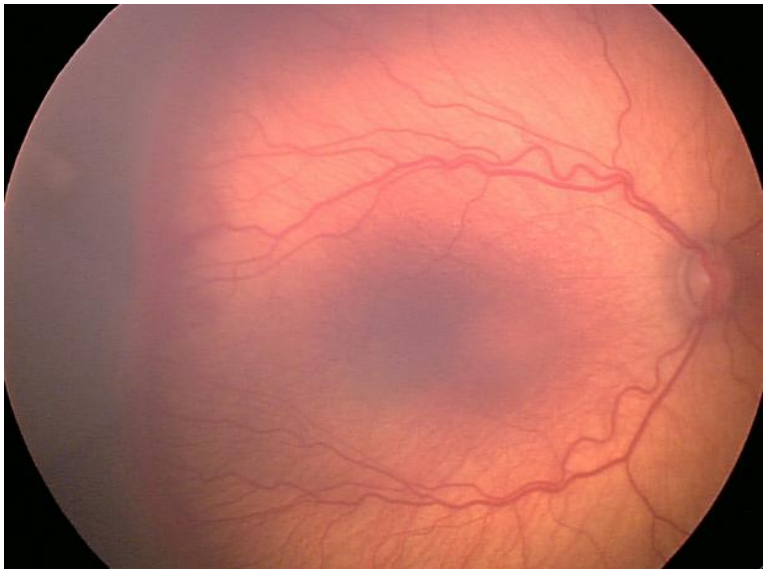

figure 2.3: non-uniformly illuminated image

- Presence of vessels that don't belong to the retina (choroids vessels), that appear in the image because of the extremely slightness of the retina in newborns.

figure 2.4: transparence of newborns retina

- Fuzzy areas in the image

figure 2.5: fuzzy image

RetCam images present, in general a low level of resolution that makes challenging their automatic analysis.

The main difference between RetCam and a normal fundus camera is the field of view. RetCam images have a wide field of view (120°-130°) whereas fundus camera images usually have a field of view which doesn't exceed 60°. Beyond that, infants eyes present a very flimsy retina that is extremely transparent and through which choroidal vessels are often easily confused as retinal vessels.

In order to monitor newborns retina and to diagnose the presence of ROP, its stage and its acuteness, the possibility of examining images of a large area of the eye is indispensable, but this leads to quality loss in the acquired images.

Even in the best RetCam images the quality is not comparable to a standard fundus camera image, because of the different field of view and because of the strong difference between a mature adult eye and an infant eye. In fact imaging retina in premature infants is considerably more challenging than in adult subjects. Factors such as cloudy media, small pupils, uneven illumination from an annular light source, and difficult in examining an

extremely preterm infant combine to limit the quality of the images obtained [10].

The images below show the difference between a fundus camera image of an adult retina and a RetCam image acquired from an infant to understand the difficulty in automatic or non-automatic analysis of RetCam images.

figure 2.6: fundus camera image of an adult eye

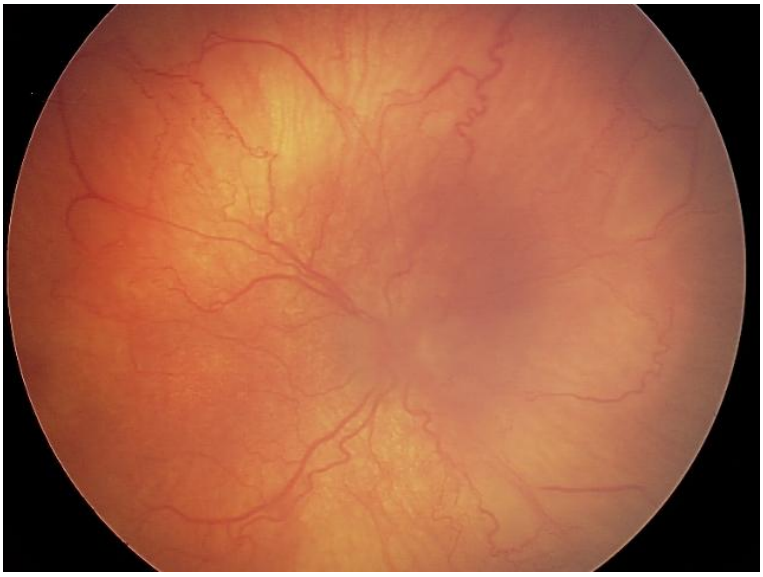

figure 2.7: RetCam image of an infant eye

2.3 RETCAM IMAGES IN PREMATURE NEWBORNS

Despite of all the difficulties related to RetCam images analysis, progress in image analysis promises to revolutionize specific areas of medical practice and enable the quantification of signs that hitherto have been described only in a qualitative manner. Improved image acquisition and automated analysis have the potential to improve the cost effectiveness of retinal screening programs.

In ROP Plus Disease is an indicator of ROP severity and may present a constellation of signs. The most important are an increase in retinal arterial tortuosity and venous dilation at the posterior pole, vitreous haze, and iris rigidity. Plus Disease is difficult to quantify in an objective and reliable way, since the diagnosis is based on a 20-years-old reference photograph.

Preplus Disease, described as vascular abnormalities at the posterior pole that are insufficient to diagnose plus disease but that demonstrate more arterial tortuosity and venous dilation than normal, was added to the classification of ROP in 2005.

The diagnostic challenge that this disease presents is illustrated by the lack of interobserver repeatability of disease presence and absence by a panel of expert readers.

The main purpose of this work is to overcome the inherent inaccuracies in qualitative evaluation using automated techniques. The final aim is to obtain an automated technique able to calculate in a reliable way, parameters as arterial tortuosity or venous dilation, that are determinant to diagnose in an objective and efficient way.

The first step to a completely automated analysis of RetCam images is the automated tracking of the retinal blood vessels basing on RetCam images analysis and elaboration.

In the following chapters we will describe our methods, we will show the results we obtained and we will discuss the improvement that can still be made and the further aims.

CHAPTER 3: MATERIAL AND METHODS

As explained in the previous chapter, the aim of this work is the realization of a suite of algorithms able to automatically track the retina blood vessels network in premature newborns eyes elaborating RetCam images.

This work is based on a set of 40 images acquired from preterm newborns. The RetCam used to collect the images (Clarity Medical System, California) has a 130° field of view and the fundus camera has a VGA sensor. The resolution is 640 X 480 pixels.

First of all, six images have been selected as example of the different kinds of images belonging to our set. These images are fundamental because we will use them as training set during the classification process. They also represent a pattern of each kind of image that the software will probably have to analyze, in fact we chose six mages having different characteristics in color, brightness and definition.

The following images are the six selected from our training set:

figure 3.1 :image 1

figure 3.2: image 2

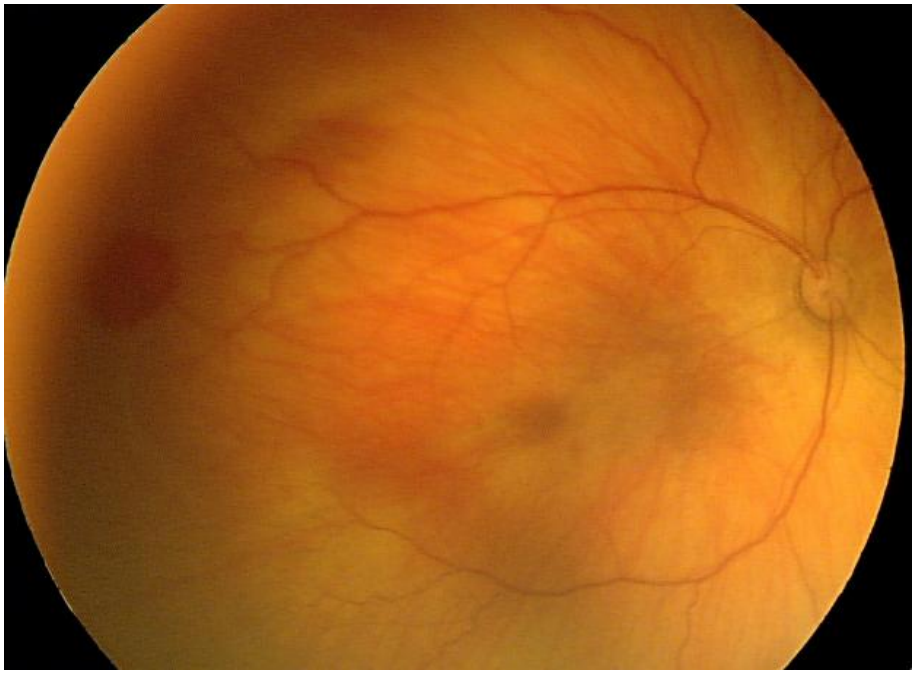

figure 3.3: image 3

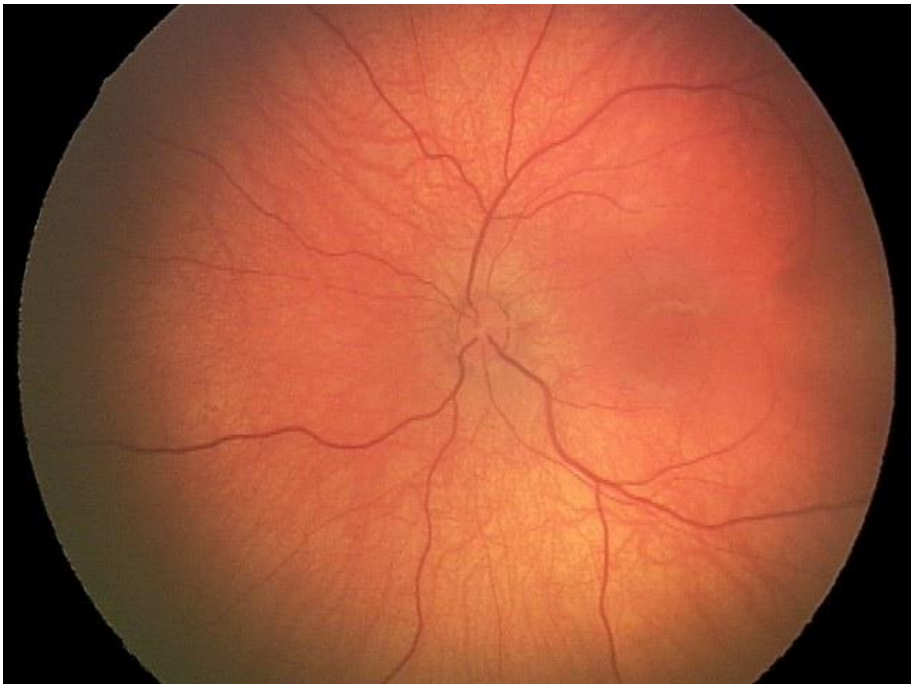

figure 3.4: image 4

figure 3.5: image 5

figure 3.6: image 6

Considering the low contrast and the non-uniform illumination of the images, preprocessing step, through some enhancement functions and through filtering, is fundamental in our analysis.

Several kinds of filters have been tested on the chosen images. The filtering aim is to enhance the structures that more likely belong to the blood vessels network.

Starting from the filtered image, several segments have been collected and saved in a structure through a segmentation process. Each segment can be or not a part of a blood vessel.

The next step has been the classification process. The aim of this step is to classify each segment of the structure previously created as vessel segment or background segment. We used a support vector machine previously implemented by another group. The more difficult and important part of the classification step has been the decision of the features that can bring to a correct classification.

After classification we obtained binary images, having white blood vessels and black background.

3.1 PREPROCESSING

As underlined previously, retina images of newborns acquired using RetCam present several problems that make difficult their analysis. The most serious are the lack of illumination uniformity and the low contrast.

The consequence is that these kinds of images have to be accurately preprocessed before the elaboration and classification process.

From now on, in this work we will consider only the green channel of each image because it assures a higher contrast between vessels and background.

We then preprocessed the images by an enhancement function which purpose is to reduce the variation of illumination and to amplify the contrast between blood vessels and background.

3.1.1 ENHANCEMENT FUNCTION : ILLLUMINATION

The function we used separates the input image in several blocks and calculates the intensity mean for each block. The brighter areas of the image will have high value of the intensity mean whereas the darker areas of the image will have a low value of the intensity mean.

To avoid discontinuity among the different blocks we applied an interpolation function which assures continuity in the image.

Then, the intensity mean of each part is subtracted from each block of the original image. In this way a high value will be subtracted from the brighter parts of the image and a low value will be subtracted from the darker parts. The result will be an image where there aren't strong variations of brightness among different zones.

3.1.2 ENHANCEMENT FUNCTION : CONTRAST

The same function also has the objective to enhance the contrast between background and structures that can possibly belong to the blood vessels network. The enhancement has been obtained dividing the image in square blocks and applying a Matlab function (function histeq) to each block. This function performs histogram matching and it changes the histogram of the

image given as input and makes it the more analogous as possible to a histogram which shape is chosen by the user. We chose a histogram having a Gaussian shape, with mean equal to 0.4 and standard deviation equal to 0.2.

On one hand this step is unavoidable because it improves filtering performance and it emphasizes the structures we are interested in. On the other hand, it also enhances the noise. A median filter applied just after the enhancement process reduces the problem of the noise.

figure 3.7: green channel of the original image

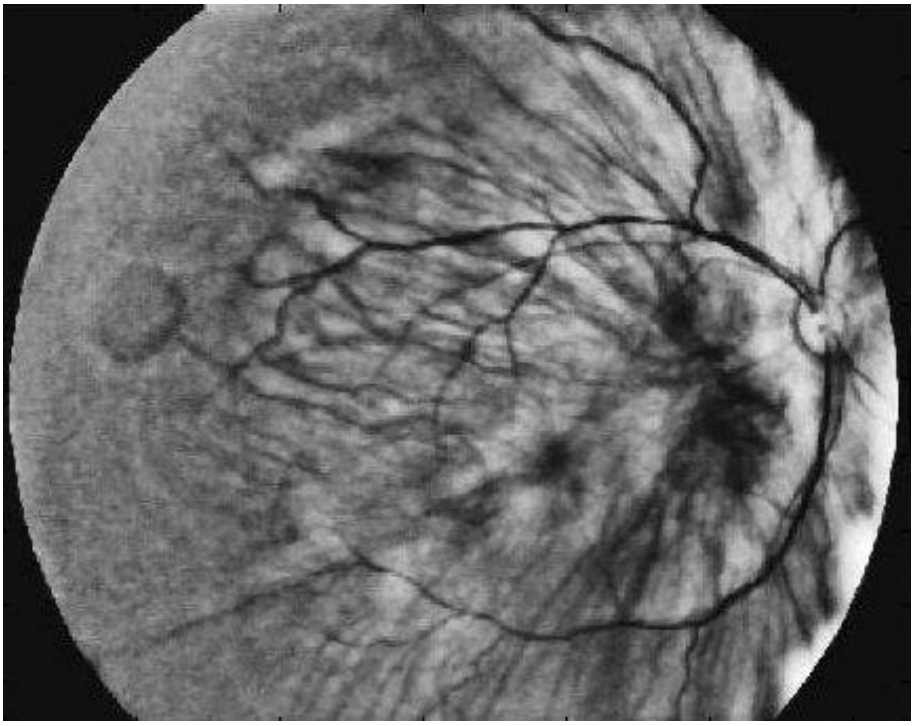

figure 3.8: enhanced image

3.2 FILTERING

This is one of the most important steps of the work because all the classification process will base its decision on information that largely come from the filtering result.

In order to achieve an accurate vessel-tree reconstruction, filtering procedure is a very important preprocessing step.

Starting from the image previously enhanced we applied several kinds of filters:

- Hessian directional filter on directional image;
- Hessian directional filter on non-directional image;
- LOG directional filter on directional image;
- LOG directional filter on non-directional image;
- LOG non- directional filter on directional image;
- LOG non-directional filter on non-directional image;

In this work, we applied all the filters mentioned above to the six chosen images to evaluate which one of the filters performed better.

3.2.1 DIRECTIONAL IMAGE

As just explained, in this work we tried different kinds of filters to find the one which better enhanced the blood vessels structure. We also tried to apply these filters to 2 different kinds of images:

1) The image after the application of the enhancement function previously described (non-directional image);
2) The image after the application of the enhancement function previously described and after the application of a sequence of filters that emphasize any threadlike structure along a specific direction. The filter has been applied along seven different directions (seven different angles thetas) obtaining seven different images having each one a specific direction emphasized. The final image (directional image) is the mean of all these seven filtered images

Using the directional image, the directional information present in an image is used. Following the idea of an approach already developed in literature, but simplifying the method to obtain it, the image is first decomposed into a set of directional images (seven different angles thetas so seven different directional images). Each directional image contains line-like features in a narrow directional range. Then, a function that enhances line-like structures along the specific direction of the image is applied to each directional image and finally the enhanced directional images are recombined to generate the output image with enhanced vessels and suppressed noise.

We created the directional images and applied our filters also to these kinds of images because they should have two important advantages: one is, noise in each directional image will be significantly reduced compared to that in the original one due to its omni-directional nature; the other is, because one directional image contains only vessels with similar directions, the Hessian eigenvalue calculation in it is facilitated.

3.2.2 HESSIAN DIRECTIONAL FILTER

Hessian-based multiscale filtering has been proposed in several vessel enhancement and preprocessing approaches. One advantage of the

approaches in this category is that vessels in a large range of diameters can be captured due to the multiscale analysis. In these methods, an input image is first convolved with the derivatives of a Gaussian at multiple scales and then the Hessian matrix is analyzed at each pixel in the resulting image to determine the local shape of the structure at that pixel. The ratio between the minimum and the maximum Hessian eigenvalues is small for line-like structures and should be high for blob-like ones.

The drawbacks of Hessian-based approaches are that they are highly sensitive to noise due to the second-order derivatives and that they tend to suppress junctions [5].

In this work we used a multiscale Hessian filter, considering six different scales (0.5; 1; 1.5; 2; 2.5; 3) related to six different vessels widths. In fact the image contains vessels of different widths and the filter will respond in a stronger way for a small scale when it's considering a thin vessel (as capillaries), and for a large scale when it's considering a large vessel (veins and arteries). We applied the filter along seven different directions (seven different angles thetas). The filter is directional because there isn't symmetry between the x and the y components of the filter.

Once created the Hessian matrix we applied the filter along each direction and at each scale. Each pixel has six different filter responses (one for each scale). At a fixed image direction, the Hessian filter responds with different intensity to each scale and the filtering function we created keeps only one response for each pixel, the more intense.

For one of the proposed filters, we applied the multiscale Hessian filter to each of the seven directions(seven different angles theta) obtaining seven different filtering, one for each direction, where the best scale is kept for each pixel.

In order to obtain one single filtered image we so calculate the mean among the seven different filtering we had.

Other than to directional images (Hessian directional filtering on directional images), we applied this process to the non-directional images (Hessian

directional filter on non-directional images) by considering the original image instead of its seven directional components.

3.2.3 LOG (LAPLACIAN OF GAUSSIAN) FILTER

The Laplacian is a isotropic measure of the 2^{nd} spatial derivative of an image. The Laplacian of an image highlights region of rapid intensity changes and it is therefore used for edge detection[20].

Since the input image is represented as a set of discrete pixels, it's necessary using a discrete convolution kernel that can approximate the second derivatives. Because these kernels are approximating a second derivative measurement on the image, they are very sensitive to noise. To counter this the image is often Gaussian smoothed before applying the Laplacian filter.This pre-processing step reduces the high frequency noise components prior to the differentiation step. In fact, since t convolution operation is associative, the Gaussian smoothing filter and the Laplacian filter can be convolved, creating the Laplacian of Gaussian filter. Then this filter is convolved with the image to obtain the required result.

3.2.4 LOG NON-DIRECTIONAL FILTER

In this case we used a multiscale LOG (Laplacian of Gaussian) filter having a symmetric shape along the two directions x and y. This means that we didn't need to apply the filter along different directions, in fact the result would have been always the same. We so changed only the scale, applying six filters having six different scales and we chose as final filtering the best one for each pixel.

This filter has been applied both to directional (LOG non-directional filter on directional images) and non-directional (LOG non-directional filter on non-directional images) images.

3.2.5 LOG DIRECTIONAL FILTER

The other filter we used was a multiscale LOG (Laplacian of Gaussian) directional filter. The filter is directional because its shape is not symmetric

along the two different directions x and y. In the case of the LOG directional filter on directional images, we applied the six different LOG directional filters (one for each of the six scales) along seven different directions (using for every filter direction the corresponding image direction component). As described before, for each direction, the highest response among the six scales is considered. Then the mean along the different directions is calculated. In the case of LOG directional filter on non-directional images, the multiscale LOG was applied to the original image instead of its seven directional components.

The following pictures show the different results we can obtain applying each one of the filters described above to the same image:

figure 3.9: green channel of the original image

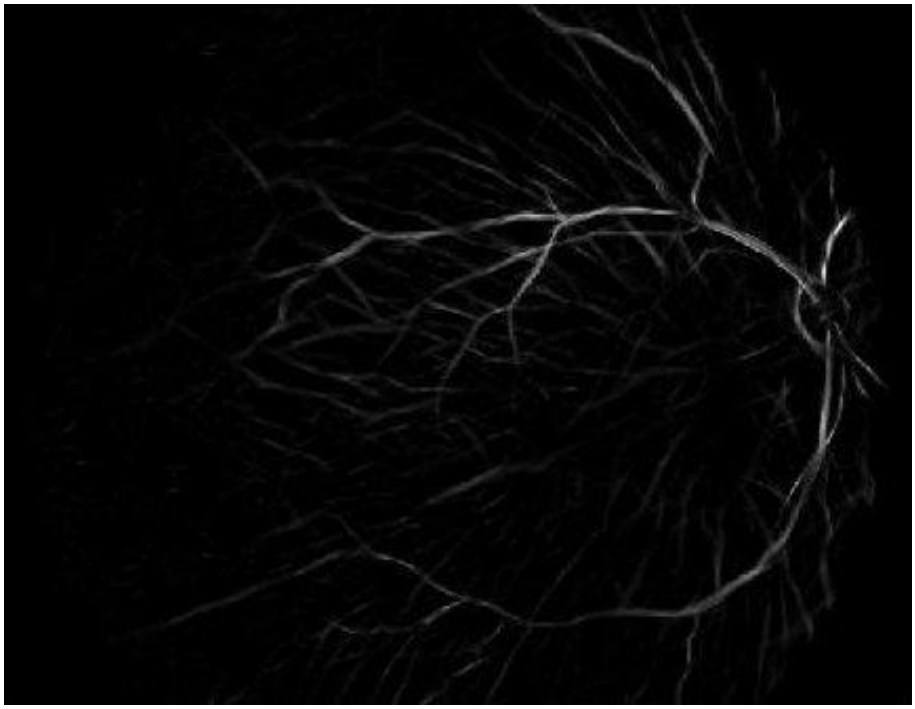

figure 3.10: Hessian directional filter on directional image

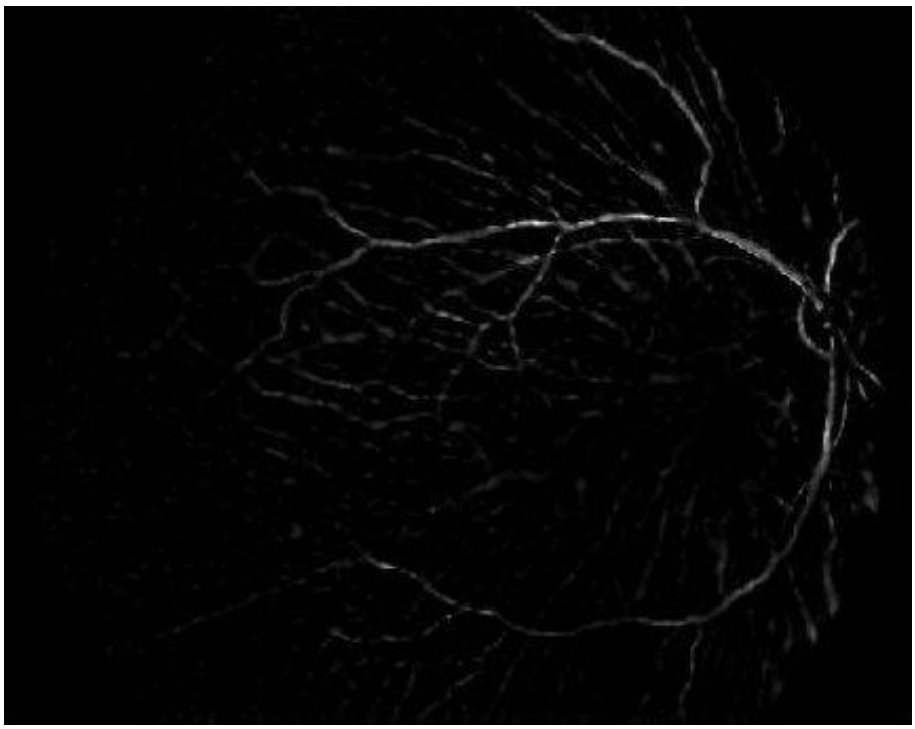

figure 3.11: Hessian directional filter on non-directional image

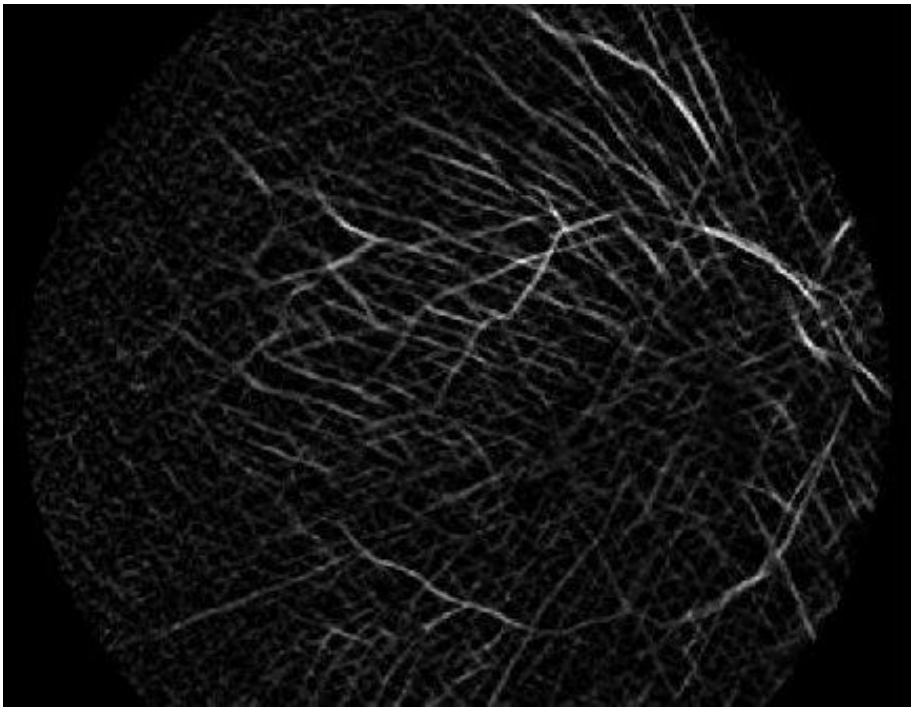

figure 3.12: LOG directional filter on directional image

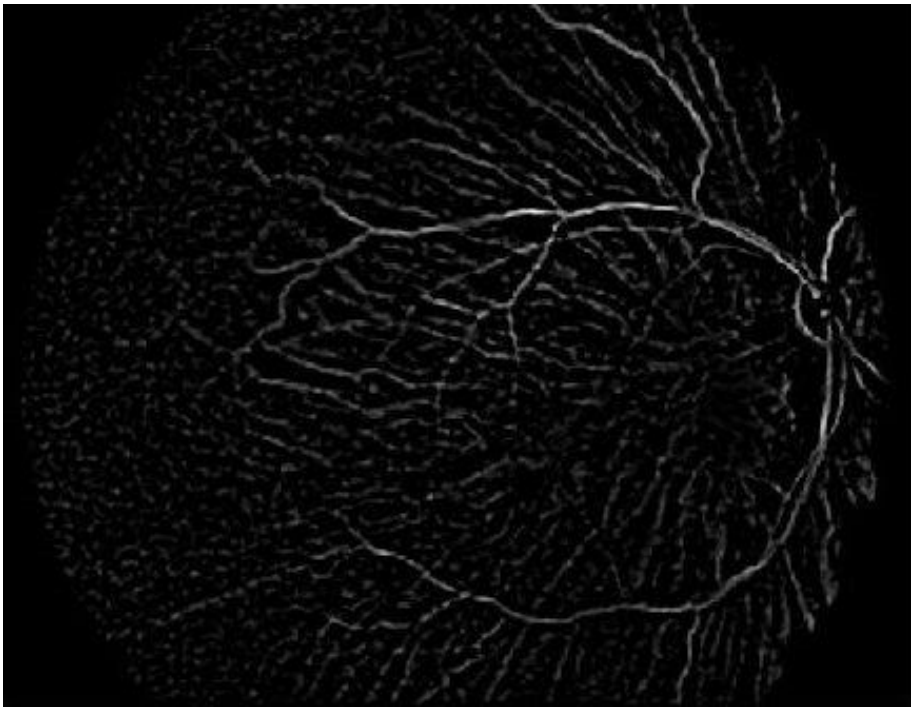

figure 3.13: LOG directional filter on non-directional image

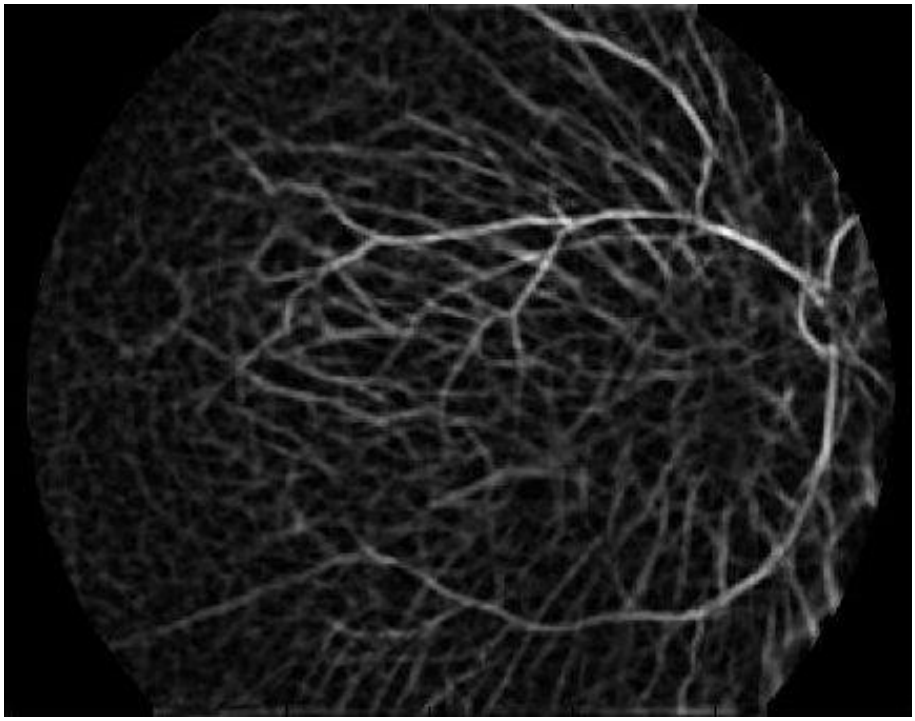

figure 3.14: LOG non directional filter on directional image

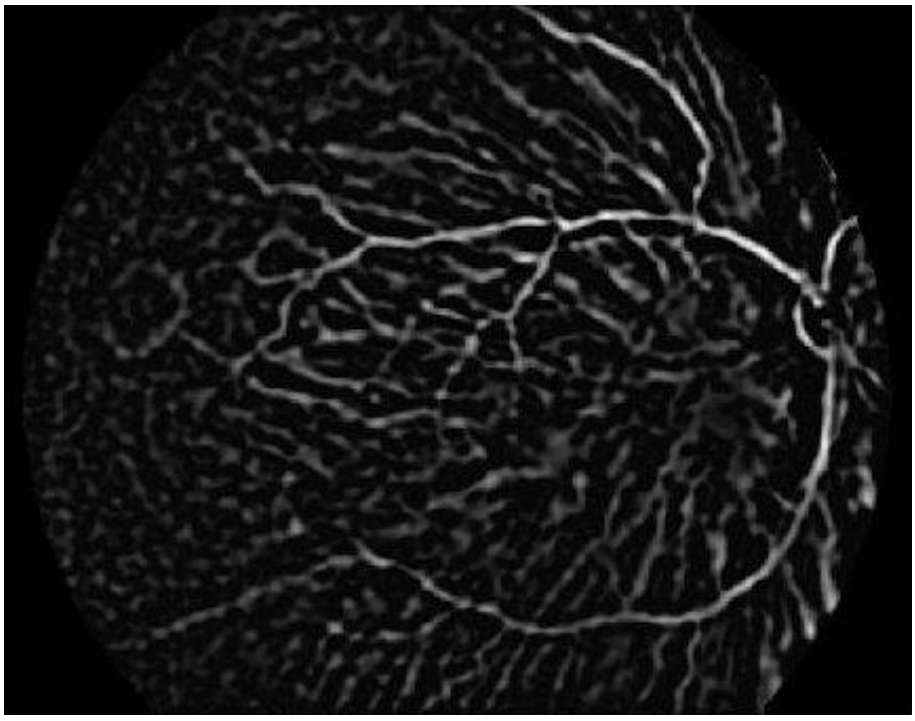

figure 3.15: LOG non-directional filter on non-directional image

These filters have been chosen for their capacity to emphasize linear structures as blood vessels. The main drawback is that they obviously

enhance also undesirable parts of the image due to the noise. In some cases the noise is excessively enhanced so we discharged two of the six possible filters and we continued our analysis basing only on the images obtained from:

- Hessian directional filter on directional images (vmD FILTER);
- Hessian directional filter on non-directional images (vm FILTER);
- LOG directional filter on non- directional images (LVD FILTER);
- LOG non-directional filter on non-directional image (LV FILTER).

We excluded the result of the LOG directional filter on directional images and the result of the LOG non-directional filter on directional images also because of the lack of possibility to discriminate between vessels and background basing on these kinds of images. Moreover, as we'll explained later, the SVM (Support Vector Machine) we used gave us the less accuracy when the classification was based on the filtering just mentioned

3.3 SEGMENTATION AND EXTRACTION OF THE AXIS NETWORK

After preprocessing and filtering each image we obtained four different possible images that could have been further elaborated (one for each kind of filters we kept).

The first step was a" skeletonization process". We used a function that realizes a local threshold and that considers as vessels all the pixels having an intensity that locally exceeds a certain value, and as background all the pixels having an intensity below this value (in fact the image after filtering is characterized by a dark background and bright line-like structures). Then the function realizes the "skeletonization process": all the structures considered as possible blood vessels are thinned in a progressive way until the point in which if thinned once more they would disappear.

This function brings to the creation of a skeleton that will be the fundamental input of the next function. The next function, basing on the skeleton previously created, builds a structure containing segments. These segments

are all the parts of the image that are considered as possible parts of the blood vessels axis network.

Actually, among these segments, there also are a lot of threadlike structures that comes from artifacts due to the noise or to the low quality of the images under study or that belong to choroidal blood vessels.

The structure containing all these segments is called “spgraph” and it is the base of the following steps.

3.4 CLASSIFICATION

After processing the images using several kinds of filters the use of a method able to discern which pixels belong to a vessel and which belong to background is necessary.

In fact the result we want to obtain is a binary image, where pixels that belong to vessels are white and pixels that belong to background are black.

3.4.1 INTRODUCTION TO SVM

SVM (Support Vector Machine) is a supervised learning method used for classification and regression. Considering, for example, a training set in which each instance belongs to one of two categories, an SVM training algorithm builds a model that, receiving as input a new instance, is able to classify it in one of the two possible categories.

As explained above, SVM is a supervised learning method which needs training and testing sets. Each example in the training set is carachterized by one “target value” (i.e. the class labels) and several “attributes” (i.e. the features). SVM will produce a model(based on the training data) which assigns to each test data its target value, receiving as input only the test data attributes [13].

Using SVM the training vectors are mapped into a higer dimensional space. SVM finds a linear separating hyperplane with the maximal margin in the higer dimensional space. Each SVM is characterized by a function called the kernel function.

The most commonly used kernels are [12]:

- Linear ;
- Polynomial;
- Radial basis function (RBF).

In this work we decided to use the RBF kernel as suggested in the svm guide and we proceeded in this way:

- We checked the data, assuring that they were in a SVM format and we scaled them;
- We considered the RBF kernel;
- We used cross-validation to find the best parameter C and γ;
- We used the best parameter C and γ to train the whole training set;
- We tested the functionality of our classifier.

The two main function of this SVM (called LIBSVM) are [13]:

1)model=**svmtrain**(training_label_vector,training_instance_matrix);

2)[predicted_label,accuracy,decision_values/prob_estimates]=

svmpredict(testing_label_vector,testing_instance_matrix,model);

Training_label_vector in svmtrain is an m by 1 vector of training labels.

Training_instance_matrix in svmtrain is an m by n matrix of m training instances with n features.

The 'svmtrain' function returns a model which can be used for future prediction.

Testing_label_vector in svmpredict is an m by 1 vector of prediction labels. If labels of test data are unknown, it is an m by 1 vector of any random values.

Testing_instance_matrix in svmpredict is an m by n matrix of m testing instances with n features. Model in svmpredict is the output of svmtrain.

The function 'svmpredict' has three outputs. The first one, predict_label, is a vector of predicted labels. The second output, accuracy, is a vector including accuracy (for classification), mean squared error, and squared correlation coefficient (for regression).

In this work we used LIBSVM for classification so we had to create our training_label_vector and our training_instance_matrix to train the support

vector machine and a testing_instance_matrix for each image to classify, establishing which features characterize each image.

3.4.2 MANUAL TRACKING

So far we described several steps that aim to automatically elaborate a RetCam image to obtain the retinal blood vessels tracking.

Since now our elaboration process built a structure containing several segments that we will classify as vessels or not vessels.

To understand the efficacy and the reliability of our classification process we needed to compare the result we obtained to a ground truth of the same image. The manual tracking is considered the real retinal blood vessels network and the software performance has been evaluated basing on the manual tracking.

These ground truth images are essential for performing a quantitative analysis of the algorithms result. Both the ground truth images and the final result images are binary images, so they can be compared easily.

In this work we manually tracked the retina blood vessels network of the six images previously showed and of other 14 images chosen from our testing set.

These 20 binary images represent the reference point we used to establish the sensitivity and other parameters that let us to evaluate the performance of our elaboration system.

The 6 binary images are fundamental in the training step of the classifier we used, in fact the “training_label_vector” (one of the inputs of the function svmtrain described above) by which was possible the creation of the model used by the svm to classify the other images of our set, was built using these manual tracked images.

The images below show an image from our testing set and its manual tracking.

figure 3.16: green channel of the original image

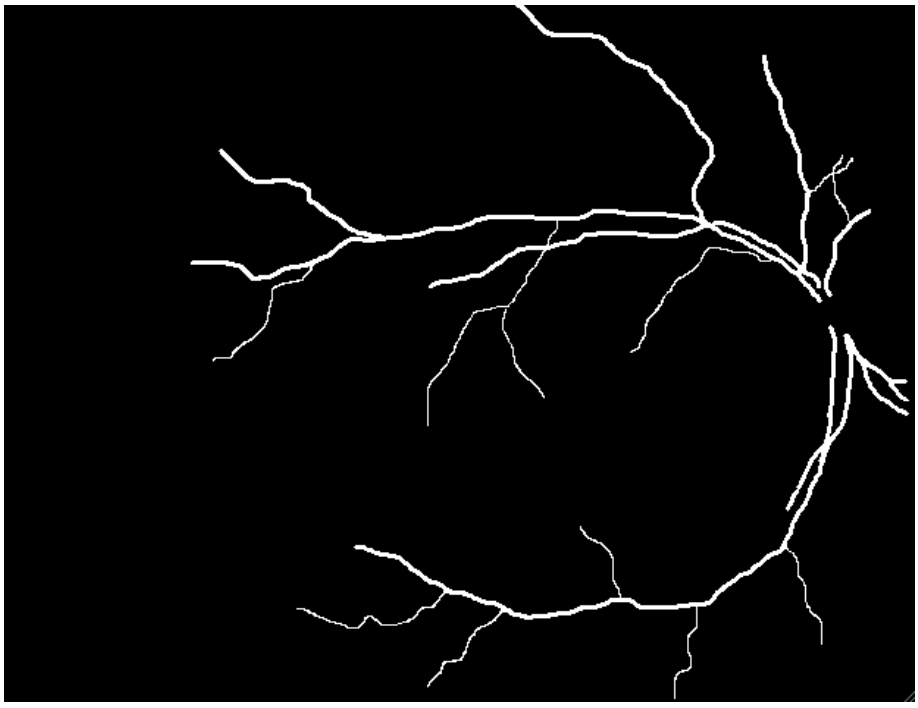

figure 3.17: manually tracked image

3.4.3 TRAINING

The training process of our SVM (structure vector machine) has been made starting from the set of 6 images manually segmented. In particular, we calculated for each image a series of segments that could belong or not to the blood vessels network (these segments are contained in the structur spgraph, see paragraph 3.3). The training process was based on the function svmtrain: the result was a model used by the classifier to classify the other images of our set. As already explained we decided to exclude LOG directional filter on directional images and LOG non-directional filter on directional images results, so we considered only the segments of the 6 images calculated after LOG directional filtering on non-directional images (LV filter) or after LOG non-directional filtering on non-directional images.

For each image we calculated a m x 1 vector, where m is the number of segments that could possibly belong to the blood vessels network. The training was based on a M x 1 vector, where M is the sum of all the segments of the 6 images used for the training. This means that our training step was based on a total of 2792 segments, when applied to the images filtered by th LOG directional filter on non-directional images (LVD filter) and on a total of 1772 segments when applied to the images filtered by the LOG non-directional filter on non-directional images (LV filter). Basing on the manual tracking of the 6 images, we could assign to each segment a value (+1 or –1) if the segment effectively belonged to the blood vessels network (value +1) or not (value -1).

To each segment were also associated several features. The combination of the features associated to each segment and the value (+1 or -1) allowed us to train the SVM. We did it for the images filtered by the LV filter first, and then for the images after LVD filtering, obtaining two different models (LV model and LVD model).

3.4.4 FEATURES

We decided to calculate the features necessary to our classifier considering each segment contained in spgraph. As explained above, spgraph contains the segments in the image that could most likely belong to the blood vessels network and the coordinates x and y of each pixel in each segment. The segments are part of the image that can belong to the vessels or not and the aim of the classifier we used is to classify each segment of spgraph as vessel or not vessel basing on the model built from the training set and on the features we provide for each image that has to be classified.

These features are saved in a matrix having m x n dimension, where m is the number of segments of each image and n is 8*15+8+8=136. 15 is the number of pixels that describe a profile calculated for each segment on several scales (six different scales), on the final filtered image and on the green channel of the original image.

The profiles are calculated in this way:

- The function implemented elaborates each segment of the image, considering all of them through a cycle for;
- The perpendicular direction of each segment is calculated;
- Then, for each pixel of the segment considered, a 15 pixels length profile along the direction previously defined (the perpendicular direction of each segment) is calculated;
- In the final step the mean among all the profiles of a single segment is calculated along the direction of the segment and we consider this mean as the characteristic profile of that segment.

The typical profile of a blood vessel structure:

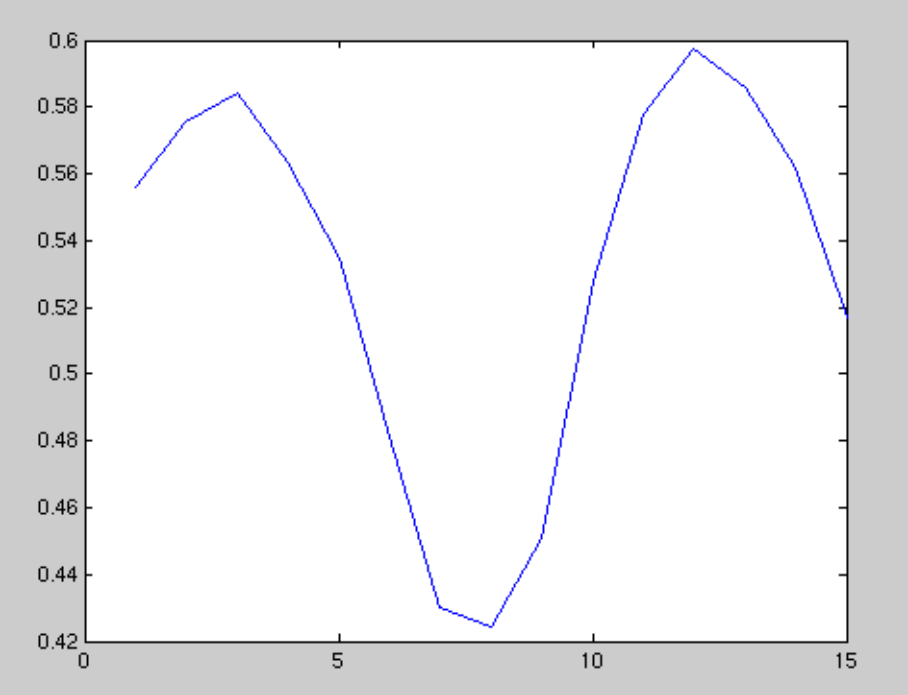

figure 3.18: blood vessel typical profile

The matrix of features is built calculating these profiles for each segment on each filtering scale (in fact our filters are multiscale filters, and we used six different scales representing six different vessels widths), on the final filtered image and on the green channel of the real image. We show above some example of the kind of images in which each profile of each segment has been calculated.

figure 3.19: green channel of the original image

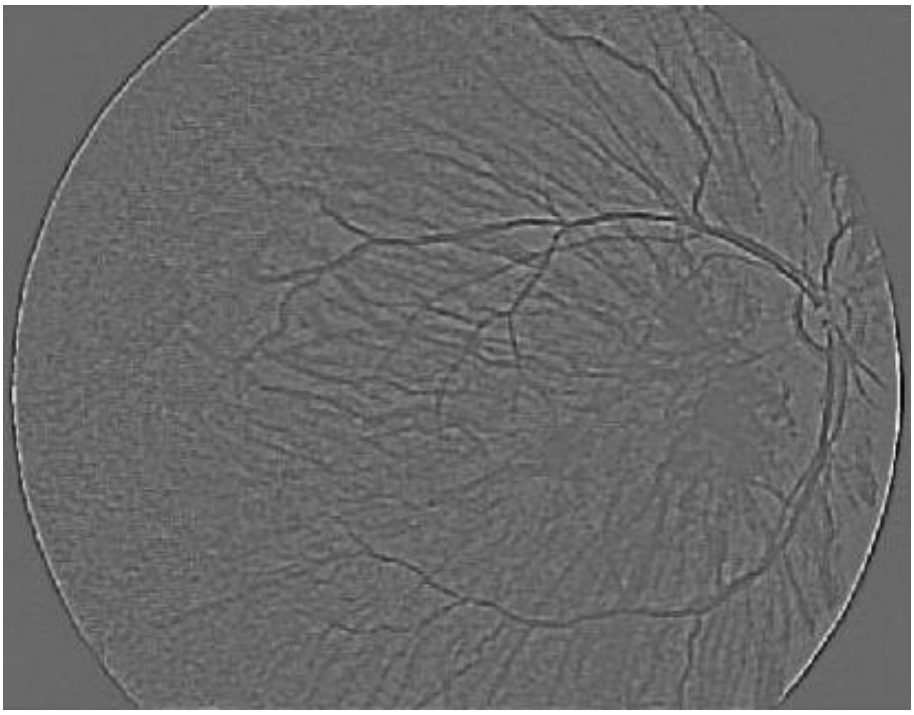

figure 3.20: LVD filter, scale 0.5

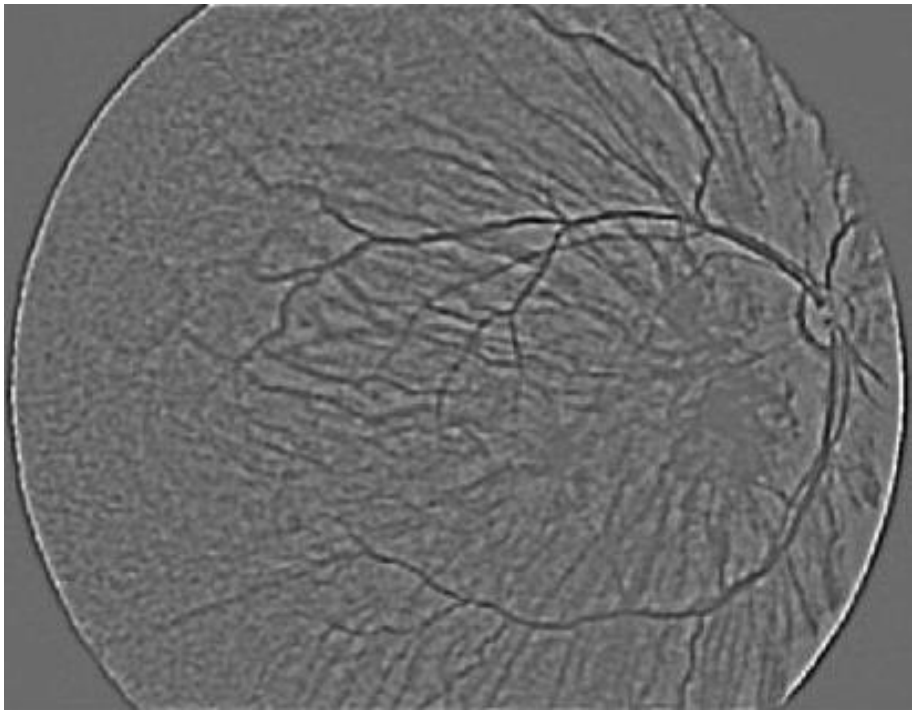

figure 3.21: LVD filter, scale 3

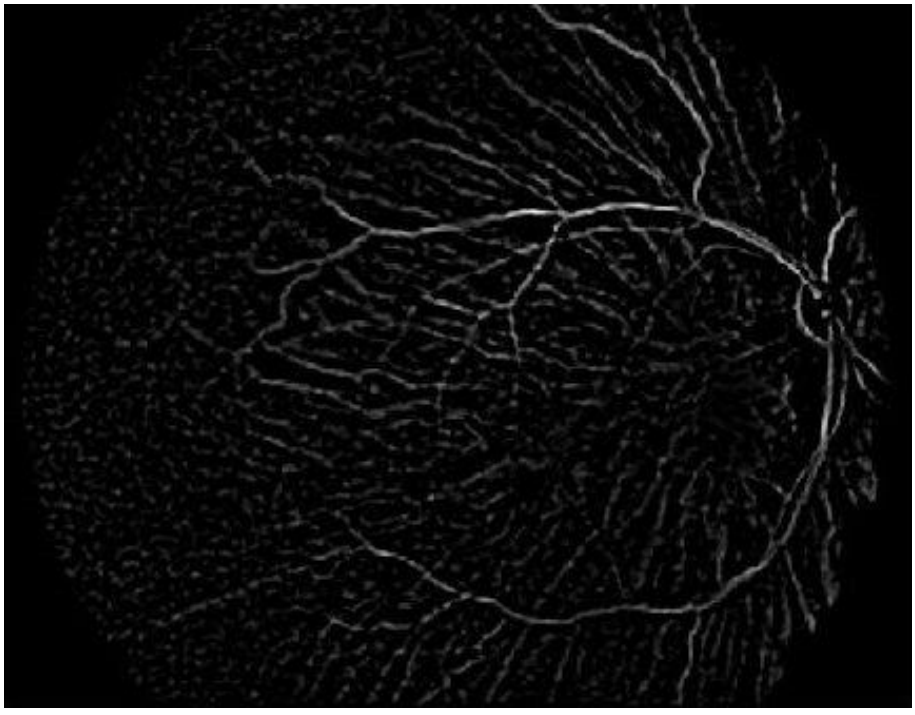

figure 3.22: filtered image

We also calculated the mean of the intensity of each segment and its standard deviation. We did it for each of the six scales, for the filtered image

and for the green channel of the original image. This means that we had to add 8 columns (the 8 means) and then other 8 columns (the 8 standard deviations) to our matrix of features.

As explained above for each image we obtained a m x 136 matrix of features where m indicates the number of segments contained in the structure spgraph of that specific image.

This matrix was calculated for each image that had to be classified and given as “testing_instance_matrix” input to the function svmpredict.

The same matrix has been calculated for each of the six images that we chose since the beginning in order to build our training_instance_matrix. For each image the matrix has been calculated basing on the Hessian directional filter on directional images filter bank and filtering result, on the Hessian directional filtering on non-directional images filter bank and filtering result, on the LOG directional filtering on non-directional images filter bank and filtering result and on the LOG non-directional filtering on non-directional images filter bank and filtering result. Then a big matrix of features has been created for each kind of filtering. Each big matrix was formed connecting one matrix of features calculated from an image under the matrix of features calculated from the other image and the six matrices of features have been unified for each filtering.

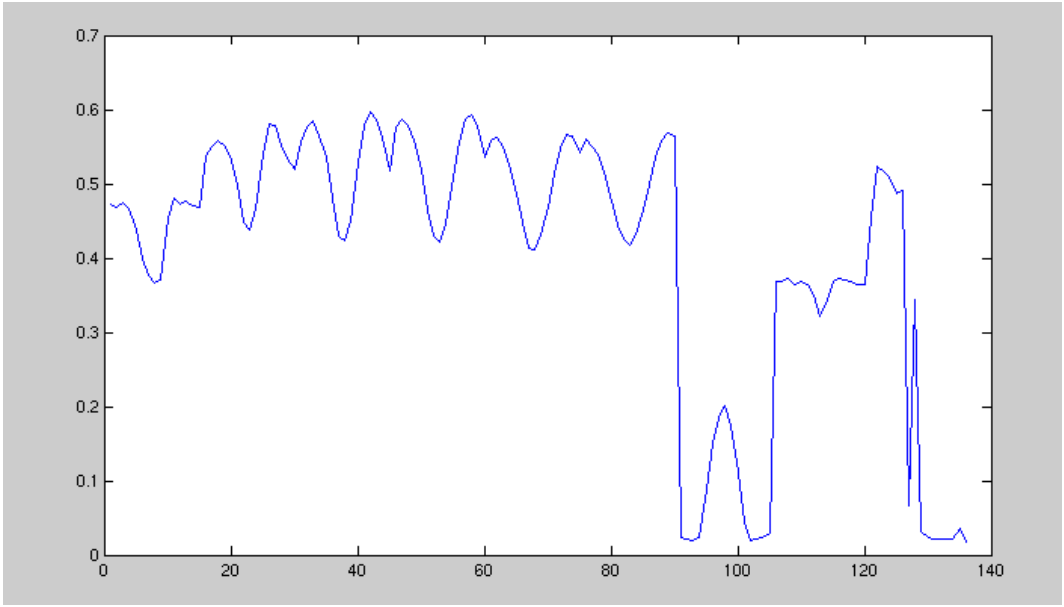

figure 3.24: row of the matrix of features which refers to a segment of the structure spgraph that actually is a blood vessel

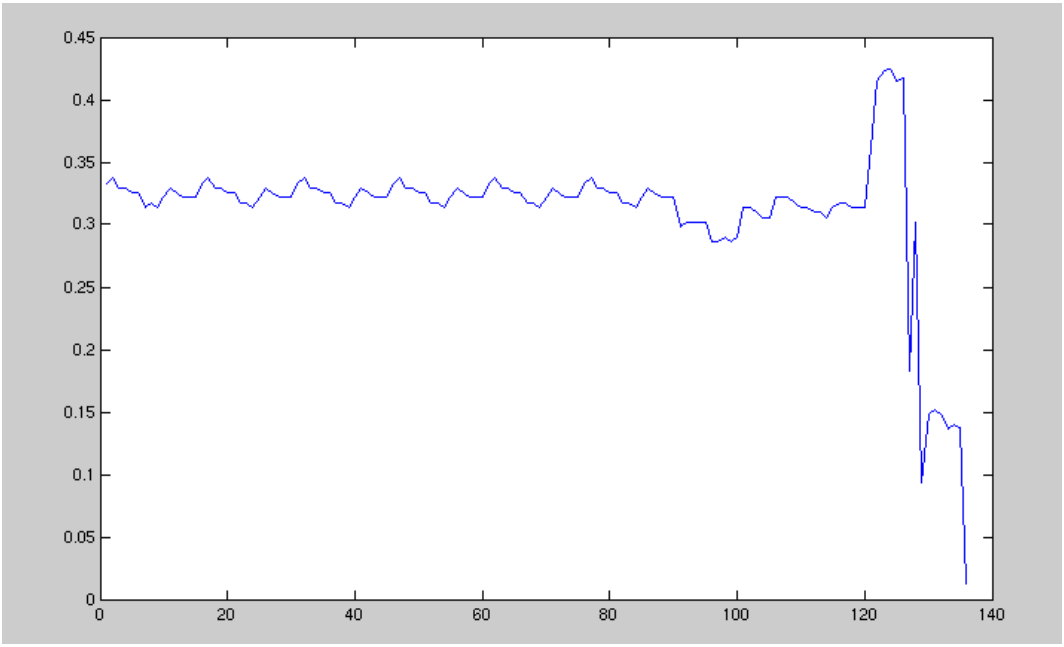

figure 3.25: row of the matrix of features that refers to a segment in spgrah that is noise

3.5 CROSS VALIDATION ACCURACY

The different filtering used are four, so we created four big matrices that represented the “training_instance_matrix” inputs for the function svmtrain, allowing us to build four different possible models. As suggested by the LIBSVM guide [12] we calculated the cross validation accuracy (number of folds=5) related to each model and the parameters *c* and *g* that maximize it.

We realized a function that calculated the cross validation accuracy associated to the parameters *c* and *g* basing on each of the four big matrices previously created. This function realized a “grid search” on *c* and *g* using cross-validation. Various pairs of *(c,g)* are tried and the one with the best cross-validation accuracy is picked. To identify good parameters the function tries exponentially growing sequences of *c* and *g* using a coarse grid first (*c* value: 2^1; 2^3; 2^5… 2^17; 2^19;and *g* value: 2^(-13); 2^(-11)…2^3; 2^5). After identifying a better region on the grid, a finer grid search on that region was conducted. The function gave us the following results:

	vmD FILTER	**vm FILTER**	**LVD FILTER**	**LV FILTER**
C parameter	32768	128	2048	512
G parameter	0.125	8	0.5	0.5
Cross validation accuracy	77.1681 %	76.9309 %	85.1011 %	82.7293 %

Table 3.1: Cross validation accuracy of each different filter

Among the different models the accuracy didn’t change significantly so we decided to try all of them and evaluate their results even expecting that LVD filter would perform in the best way.

After the SVM training and the creation of four different models, first of all we applied our classifier to the six training set images in order to evaluate if the result was consistent, then we finally applied it to the testing set images.

3.6 FINAL ESTIMATED VESSELS NETWORK

Basing on the cross validation accuracy previously calculated, we decided to focalize our attention on the classifications from LVD and LV models. As expected, both of them demonstrated to respond very well if applied to the six training images.

We then applied both the classifier (based on the LV and on the LVD model) to the remaining images. We so obtained two predict vectors containing the value 1 at the i-row if the i-segment in that specific spgraph structure was classified as blood vessel and -1 if was classified as background.

Our aim was to obtain a binary image having a black background and a white blood vessels network, so we realized a binary image for each LV and LVD classification, for each image.

Then, for each image, we created a binary image that contained only the blood vessels recognized at the same time from both the LV and the LVD models (and-image) and a binary image that contained the sum of all the blood vessels belonging to the LV classification and all the blood vessels belonging to the LVD classification (or-image). Intuitively, the blood vessels contained in the and-image have a very high probability to effectively belong to the blood vessels network we're trying to reconstruct. Starting from this basic network, we implemented a function that adds to the and-image all the segments recognized as vessels in the or-imagethat have at least an edge in common with a segment of the and-image. In this way the information of the two classifications (based on the LV and LVD models) were preserved and most of the small and isolated segments, caused by noise or artifacts, eliminated.

The final step has been the addition to this network of the segments contained in the original spgraph structure the touched the current neytwork on both extremities. In this way we could link the part of the blood vessels network that were disconnected because the classifier didn't consider as blood vessels the segments which unified them.

The final results of this sequence of step is a binary image, having a black background and a white blood vessels network.

Qualitative and quantitative results of our method will be presented in the next chapter.

CHAPTER 4: RESULTS

As explained in the previous chapter, before choosing to combine the LV-filter (LOG directional filter on non-directional images) with the LVD filter (LOG non-directional filter on directional images), we had 4 possible classifier to use, based on 4 different filtering. We tested all of them on the training set images and, as expected, they all responded with high accuracy (in fact all the models were built on that 6 images).

	IMAGE 1	**IMAGE 2**	**IMAGE 3**	**IMAGE 4**	**IMAGE 5**	**IMAGE 6**
LVD ACCURACY	88.5329	91.8605	88.4615	82.4	87.6289	88.0466
LV ACCURACY	92.3567	89.8551	89.0244	87.3239	85.1613	89.8761
VmD ACCURACY	91.1828	94.1176	93.9633	78.8644	86.7089	79.7564
Vm ACCURACY	88.6792	91.7808	92.1986	82	92.437	81.5673

Table 4.1 : classifier accuracy when applied to the training set images

Basing on the cross validation accuracy related to each of the 4 possible models and on some qualitative results, we decided to proceed our elaboration considering only LV and LVD models. Starting from these 2 results and applying some other steps to combine them (see chapter 3, paragraph 6) we finally obtained our binary images, representing the blood vessel network of the image under exam, calculated in a completely automated way.

To quantitatively evaluate the performance of our method, it was necessary creating ground truth images manually by drawing lines on vessels of original images. We did it for 20 images.

Both the result of our elaboration process and of the manually tracked images are binary images, in this way it has been possible to evaluate if each pixel in the image we obtained after elaboration is a:

- True positive (TP) = pixel correctly detected as vessel;
- False positive (FP) = pixel incorrectly flagged as vessel;

This is a fundamental step in order to calculate the performance of our method. In particular, we calculated 3 parameters:

1. T = number of total white pixel in the manual tracked image;
2. Sensitivity = sensitivity has been evaluated as the percent fraction of the length of correctly tracked vessels over the total length of the ground-truth vessels The following images show how our suite of algorithms respond to several kinds of images: two images from the training-set, three good quality images from the testing set, two bad quality images from the testing set.
3. False vessels detection = false vessel detection has been evaluated as the percent fraction of the length of the false vessels over the length of all estimated vessels (not to be confused with the false detection rate).

The results we obtained for the 20 images manually tracked are reported below:

IMAGE	SENSITIVITY	FALSE VESSEL DETECTION	TOTAL OF WHITE PIXELS
0b62b723-[…]-ae3410.3	0.7423	0.1290	23137
00000E22	0.8866	0.1698	19704
00001ECE	0.5786	0.1882	15446
3d374563- […]-78d91.10	0.7380	0.2745	18280
32fcb365-[…]-efecb2.7	0.7176	0.1338	13126
62fca871-[…]-3b4eca.1	0.8570	0.0966	15395
00000R2F	0.6989	0.1116	21297
8ef6bd77-[…]-6d59.14	0.6465	0.0921	16861
32fcb365-[…]-fecb2.30	0.6334	0.1058	18884
7219d7f7.[…]-11ea.8	0.6904	0.2765	11122
062b723-[…]-3410.13	0.6376	0.1385	18025
83f6bd77-[…]-6d59.4	0.7279	0.1062	10159
32fcb365-[…]-fecb2.26	0.8022	0.1574	13733
00003351	0.6690	0.1550	23502
3683e0e9-[…]-6cb7.46	0.9714	0.4110	11702
24084b0f-[…]-fdbfb.30	0.9118	0.4882	12246
0420001-[…]-515e.22	0.6873	0.1978	17862
ad9068f2-[…]-4676.8	0.5455	0.1124	19150
b72c1197-[…]-2236.38	0.8003	0.5216	13152
d85470ae-[…]-74bc5.1	0.5774	0.0761	18358

Table 4.2 : Sensitivity, false vessel detection and number of pixels in the ground truth image calculated for 20 images of the testing set

	SENSITIVITY	FALSE VESSSEL DETECTION
MEAN	0.7259	0.1971
LOWEST VALUE	0.5455	0.0761
HIGEST VALUE	0.9714	0.5216

Table 4.3: Mean, lowest value and highest value of the sensitivity and of the false vessel detection calculated on the 20 images manually tracked

	SENSITIVITY	FALSE VESSSEL DETECTION
MEAN	0.7141	0.2106
LOWEST VALUE	0.5455	0.0761
HIGEST VALUE	0.9714	0.5216

Table 4.3: Mean, lowest value and highest value of the sensitivity and of the false vessel detection calculated on the 14 images of the testing set

The following images show some examples of the blood vessels network found from our suite of algorithms:

Figure 4.1 : Original image 1

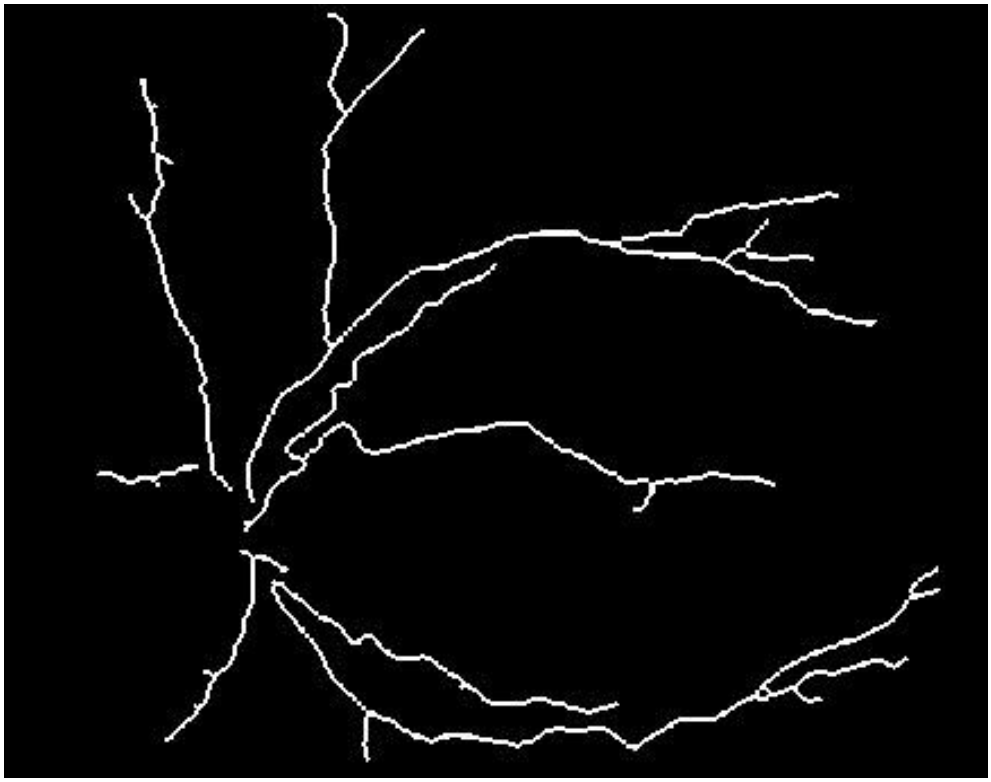

Figure 4.2 : Blood vessels network image 1

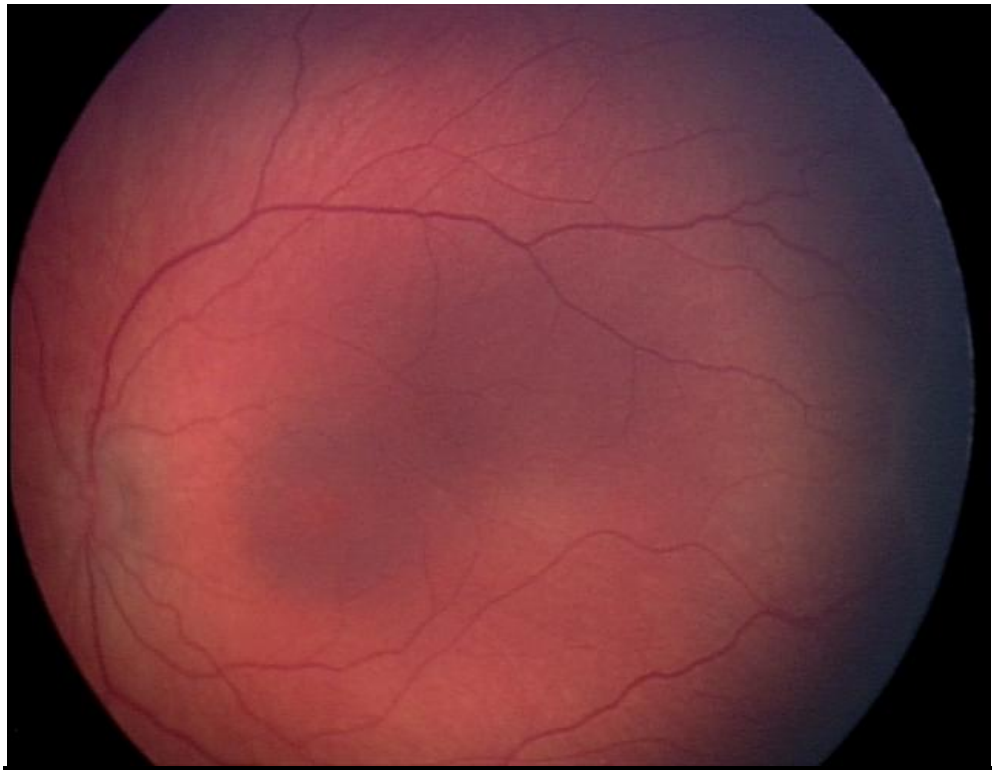

Figure 4.3 : Original image 2

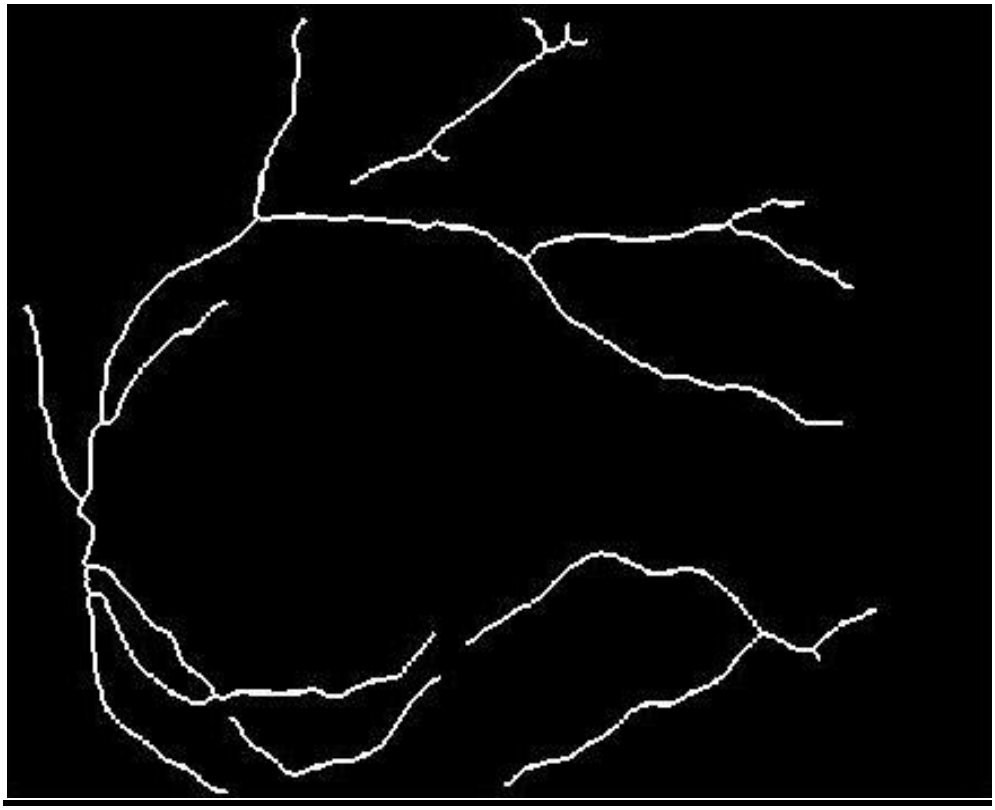

Figure 4.4 : Blood vessels network image 2

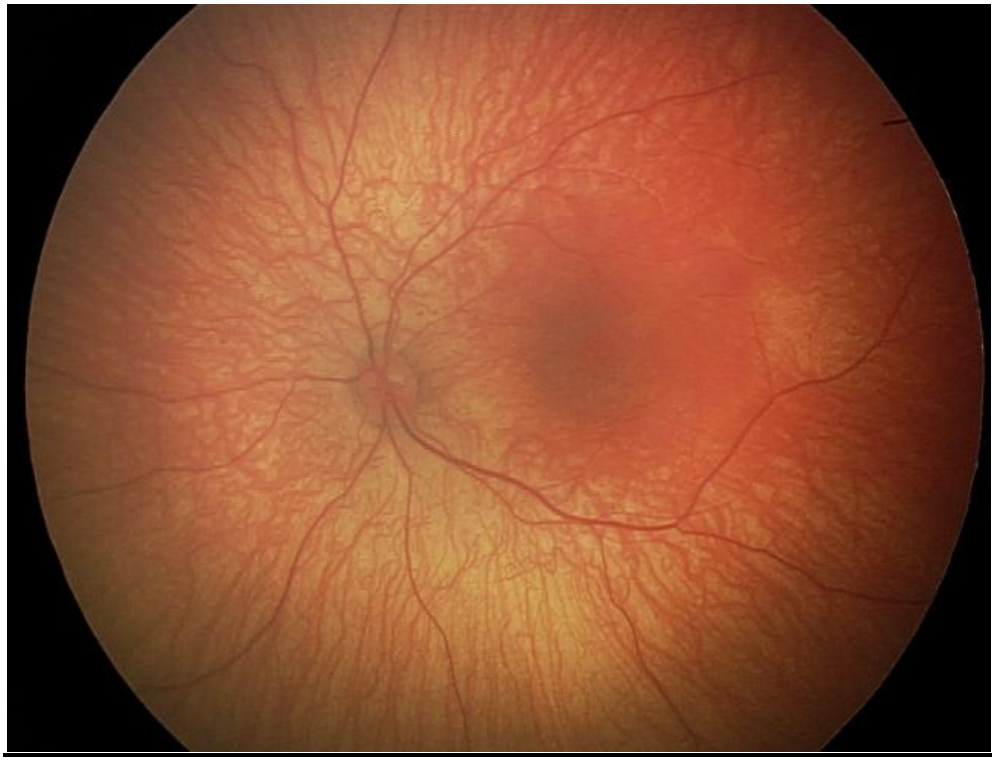

Figure 4.5 : Original image 3

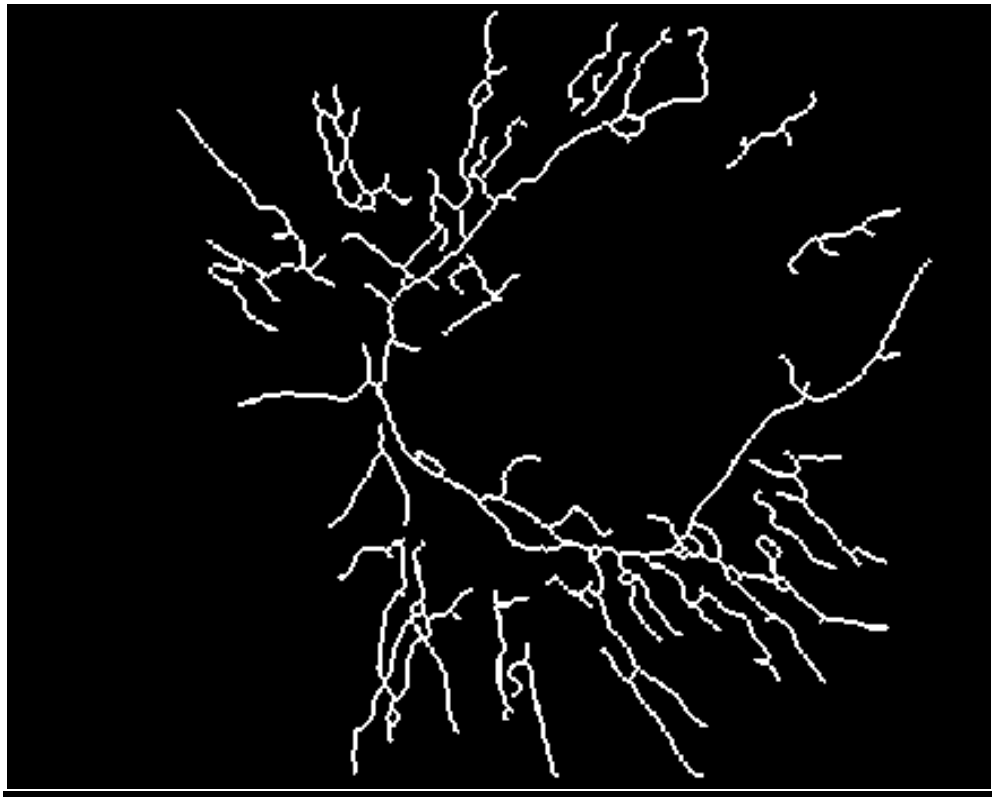

Figure 4.6 : Blood vessels network image 3

The results show that the algorithms work well in good quality images. The problem to solve Is related to those images where choroids vessels are strongly evident, because they’re recognized as retinal blood vessels by the system, worsening the performance.

CHAPTER 5: DISCUSSION AND CONCLUSIONS

5.1 DISCUSSION

Plus disease is severe dilation and tortuosity of the posterior pole blood vessels, and it is arguably the most important sign of severe retinopathy of prematurity and the major criterion for laser treatment. Plus disease is present when abnormal vascular dilation and tortuosity of at least two quadrants meets or exceeds that of the standard photograph of plus disease. Although posterior pole vascular abnormalities are frequently dichotomized into plus or not plus for purposes of classification or treatment decisions, examiners recognize that a spectrum of vascular change occurs between normal and very abnormal. Pre plus disease is defined as vascular abnormalities of the posterior pole that are insufficient for the diagnosis of plus disease but that demonstrate more arterial tortuosity and more venous dilation than normal. The presence of pre plus disease may have prognostic value and it can influence follow-up intervals or decisions to transfer infants to other hospitals. Unfortunately, judging the presence of plus disease or pre plus disease is highly subjective and prone to error. Computer assisted analysis of retinal images can potentially reduce subjectivity in the diagnosis of plus disease and optimize timing of follow-up and treatment of ROP [11].

Several researchers have attempted to establish techniques for initial diagnosis of vascular abnormalities, developing automatic vascular inspection systems to analyze physical characteristics and abnormalities of vessels by measuring size, shape and length or other network characteristic of the retina. The problem is that the algorithms just mentioned usually give a satisfactory result when applied to high quality images with low noise [10].

However, we’re considering images taken from preterm newborns, characterized by low contrast and noise, due to the lack of clarity of the eye media and the technical difficulty of obtaining images from a preterm baby. These aspects cause several problems and make considerably more challenging the realization of an automatic system able to track retinal blood

vessels in RetCam images from preterm newborns. Algorithms that have been successfully applied for adult retinal images might not work effectively enough within these limitations. It worth noticing that, in order to investigate disease and symptoms of ROP, detecting the vascular network of the retina is an unavoidable requirement. In fact this information can then be used for measurement of tortuosity and engorgement parameters.

Many previous techniques have demonstrated that they can be applied successfully on adult images. However, these methods are not sufficient to detect vessels on infant images [6]. In this work we realized a suite of algorithms able to automatically detect and track retinal blood vessels of premature newborns with a good sensitivity, analyzing images acquired using RetCam. This task represents the basic step to a more complete analysis and elaboration of RetCam images, through which important parameters like arterial tortuosity and veins dilation can be measured, giving important information to the examiners and standardizing the diagnosis and the follow-up processes [1].

Considering the low quality of the images available for our analysis, the enhancement function we created to increase the contrast between background and blood vessels and to uniform the illumination represents a fundamental step in the elaboration process.

The group of research directed by professor Alfredo Ruggeri (BioImLab), has been working on retinal images and on their blood vessels tracking for several years, but this is the first project related to RetCam images of preterm newborns so the images to elaborate presented several different and more challenging aspects compared to adults retinal images from 60° field of view fundus camera.

So we decided to enterprise an approach based on different kinds of filters, in order to realize a filtering process able to suppress part of the noise, and to enhance the components of the images that most likely form the blood vessels network we want to automatically detect and track. We also pre-elaborated each image through a filter able to enhance threadlike structure,

with the aim to obtain a filtered image where retinal blood vessels structure could be easily discerned from all the rest.

We so applied 3 different types of filters:

- Hessian directional filter;
- LOG directional filter;
- LOG non-directional filter;

to 2 types of mages:

- Green channel of the original image after application of the enhancement function we created;
- Green channel of the original image after application of the enhancement function and after the application of a bank of directional filters that emphasize line-like structures.

So, for each image analyzed, we had six different results after the filtering step. Two of the six filters applied didn't respond well, amplifying instead of suppressing or minimizing the noise, so we considered only four of the six possible results for the following steps.

After the filtering process, a classification step was indispensable, in order to discriminate the structure coming out from the filters between true vessel and entities related to noise.. We so decided to use a SVM(support vector machine) and we selected six images from our entire set of images in order to train the SVM.

We applied the four different filters to the six images selected and trained the SVM with these six images after the four different filtering, creating then four different models . The SVM bases its classification process on the model it uses, so we evaluated, through a function already implemented by the group which realized the SVM LIBSVM, the cross validation accuracy of each model, using 5 folds.

We found that the images filtered by the LOG directional filter on non-directional images (LVD filter) and the LOG non-directional filter on non-directional images (LV filter) had the best cross-validation accuracy and we use them for the classification.

The final step was the elaboration of the classifications we obtained from these two models. In fact we considered first of all, as basic network, the segments classified as blood vessels by both the models, creating a sort of “skeleton” of the complete blood vessels network of the image. Then we considered the union of the segments classified as belonging to the blood vessels network by the two models and we added only the segments that had at least one pixel in common with the basic skeleton previously created. The final step has been considering all the segments contained in the structure spgraph calculated after LV filtering and adding to our image only the segments in which both the extremities touched the blood vessel network created so far. In this way we preserved junctions and we could obtain non-interrupted blood vessels.

5.1.2 RESULTS DISCUSSION

As thoroughly explained, the realization of a suite of algorithm able to recognize and track the blood vessels network in RetCam images acquired from newborns is a challenging task. In this work we realized a completely automated method that processes the images and obtain a binary image characterized by dark background and white vessels.

Having manually tracked 20 images we computed quantitative results comparing the images after application of our algorithms and the binary images obtained after manual tracking and considered as ground truth. We so could calculate some parameters, as sensitivity and false vessel detection.

If we consider good quality images the most important components of the blood vessels network are always recognized. At the same time the structures that were enhanced by the filtering process (noise or artifacts) but that don’t belong to the blood vessels network are discharged by the classifier.

If we consider images with non-uniform illumination , usually the areas where the illumination is good is correctly classified and no blood-vessels can be detected from the dark or blurry areas.

Unfortunately the classifier couldn't work correctly in very low quality images or when choroidal blood vessels were very evident. In fact choroidal blood vessels and retinal blood vessels often present very similar gray level profiles, so the same features and the classifier aren't able to discern between the two kinds of vessels.

Nevertheless sensitivity and false vessel detection demonstrate that the algorithm first version obtains promising results.

5.2 FURTHER AIM

Considering the critical results associated to low quality images, one of the main important further aims is finding a way to discriminate choroidal and retinal blood vessels. One possibility is acting on the classifier, finding new features that discriminate better the two kinds of blood vessels. Another, more promising, possibility, is to realize a filter that is able to enhance retinal blood vessels and to exclude the vessels from the choroid.

A fundamental aim in the analysis of RetCam images acquired from newborns is to obtain data and parameters that can standardize the diagnosis and follow-up of ROP. The results of this work are only the first step through a wider an more complete objective. Calculation of tortuosity and dilation of the blood vessels are the future necessaries steps.

Since the final aim of RetCam images analysis is to provide to the clinician a device that automatically calculates the value of parameters strongly related to the real condition of the eye and to the stage of the disease, a further important step is to make possible the effective use of these kinds of software through an intuitive and easy to use interface.

5.3 CONCLUSIONS

The elaboration and analysis of RetCam images acquired from newborns represents an important task, that can bring several useful information to the clinicians and bring to a more objective and reliable follow-up of patients affected by ROP. Nevertheless, due to the low quality of the images to

analyze, it’s a challenging task. In this work we faced and tried to solve the first step, realizing a suite of algorithms that in a totally automated way, track the blood vessels network of a given images. Sensitivity and false vessels detection parameters show how the results we obtained are promising and can be strongly improved finding a way to exclude choroidal vessels from the images. Being completely automated, this method can really represents a way to standardize decisions on ROP treatment.

REFERENCES

[1] C.M. Wilson, K.D. Cocker, M.J. Moseley, C. Paterson, *Computerized analysis of retinal vessel width and tortuosity in premature infants.*

[2] P.T.H. Trucc, M.A.U. Khan, Y-K Lee, S. Lee, T-S Kim, *Vessel enhancement filter using directional filter bank.*

[3] T.Zou, *Fourier cross-sectional profile for vessel detection on retinal images.*

[4] D.Fiorin, E.Grisan, A.Ruggeri, *A web-based tool for vessel analysis in retinopathy of prematurity.*

[5] A.F. Frangi, W.J. Niessen, K.L. Vincken, M.A. Viergever, *Multiscale vessel enhancement filtering.*

[6] L. Sukkaew, B. Uyyanonvara, S. Barman, A.Fielder, K. Cocker, *Automatic extraction of the structure of the retinal blood vessel network of premature infants.*

[7] M. Vlachos, E. Dermatras, *Computerized medical imaging and graphics.*

[8] *The international classification of retinopathy of prematurity revisited.*

[9] E. Poletti, D.Fiorin, E.Grisan, A. Ruggeri, *Retinal vessel axis estimation through a multi-directional graph search approach.*

[10] D.K. Wallace, Z. Zhao, S.F. Freedman, *A pilots study using "ROPtool" to quantify plus disease in retinopathy of prematurity.*

[11] D.K. Wallace, Z. Zhao, S.F. Freedman, *Accuracy of ROPtool vs individual examiners in assessing retinal vascular tortuosity.*

[12] C.W. Hsu, C.C Chang, C.j. Lin, *A practical guide to support vector classification.*

[13] http://www.csie.ntu.edu.tw/~cjlin/libsvm/

[14] M. Vlachos, E. Dermatas, *Multiscale retinal segmentation using line tracking.*

[15] G. Marisco, *Tecniche di imaging della retina in pazienti affetti da Retinopatia della Prematurità.*

[16] http://www.anselmetti-rop.it/index.php

[17] *http://www.nei.nih.gov/health/rop/*

[18] http://en.wikipedia.org/wiki/Retinopathy_of_prematurity

[19] C. Wu, R.A. Petersen, D.K.Vanderveen, *RetCam Imaging for Retinopathy of Prematurity Screening*

[20] http://homepages.inf.ed.ac.uk/rbf/HIPR2/log.htm

RINGRAZIAMENTI

Pensavo che scrivere questa parte sarebbe stata la cosa più semplice da fare, ma non è così. I pensieri dentro di me si susseguono, le sempre presenti domande sul futuro, un libro aperto da scrivere e proprio per questo così difficile e impegnativo, non mi abbandonano neanche ora. Ma prendo un bel respiro, do un'occhiata fuori dal finestrino del treno (quanti treni ho preso in questi ultimi anni…?), e penso al presente. E penso al passato. La tesi è pronta, il momento della mia laurea è arrivato: quanta fatica, quante risate, quanti pianti, quante ore spese sui libri, quanti quaderni di appunti a cui trovare un posto.

Grazie a Diego, che mi ha aiutata, supportata e sopportata tantissimo in questi mesi, e grazie al professor Alfredo Ruggeri (che ha registrato ben la metà degli esami nel mio libretto!) che mi ha sempre appoggiata.

Grazie a Oneliù, a cui ho voluto bene dal giorno del pre-esame, per tutta la disorganizzazione che ci siamo trasmesse a vicenda, per le nostre belle figure in giro per i bar di Padova, per tutte le risate, gli abbracci, le notti a parlare e le telefonate isteriche che abbiamo condiviso.

Grazie a Ele e Marta perché gli ultimi due anni di Università rimarranno un ricordo speciale dentro di me, ed è in gran parte grazie a loro: grazie alle serate a Padova passate assieme, alle telefonate via skype, alle visite di Ele a casa mia, alle risate uniche di Marta, ai discorsi seri e meno seri che abbiamo sempre voglia di fare.

Grazie a Dé, che ha sempre cercato di farmi essere meno sbadata fino a che ha capito che non ci sono possibilità con me.

Grazie a Mauro perché senza di lui questa tesi sarebbe impaginata malissimo da me, e perché ci conosciamo da 22 anni e ne abbiamo passate tantissime assieme.

Grazie alla Glos perché da 10 anni è sempre la solita Glos, e se non l'avessi conosciuta al Liceo mi mancherebbe da morire!

Grazie a Fede per quanto mi ha fatto ridere da quando ci conosciamo, per tutti i bigliettini e i messaggi che ci siamo scritti in questi anni, per i consigli inutili ma così prezioso che ci siamo sempre dati.

Grazie a Martino, per tutte le serate passate assieme, e per i momenti indimenticabili passati in Sicilia.

Grazie a Elisa, che è stata la mia compagna di ogni avventura al Liceo, con cui ho condiviso una marea di pianti e di risate, a cui vorrò per sempre bene e che è ancora qui, a prendermi in giro come sempre.

Grazie alla Fede, che mi sopporta con tutti i miei difetti e le mie disattenzioni, per le nostre serate a chiacchierare, per il viaggio a Londra progettato da anni e mai realizzato.

Grazie alla Gaspa, perché mi ha sempre chiamata quando avevo bisogno di lei, per i nostri discorsi sull'Universo e le nostre serate glamour nell'appartamento di Padova (a letto alle 9 meno 5 di sera…), per la nostra continua ricerca del senso della vita che si conclude sempre al Fra.

Grazie a Chiara e Claudia, con le quali ho condiviso momenti pazzeschi e decisamente mai dietetici, con le quali rido sempre da morire, per tutta l'allegria che mi trasmettete e per ogni singolo momento passato assieme.

Grazie alla Vale, con cui fin da piccola ho trascorso dei momenti meravigliosi, giocando a Barbie con “Ken testa di sughero,” e chiedendoci se da grandi ci saremmo viste ancora e con cui so che posso trovarmi a parlare di qualsiasi cosa. Sempre.

Grazie a tutta la famiglia, che ho sempre sentito vicina, in particolare alla nonna, che mi ha insegnato tante cose che conservo dentro di me.

Ma soprattutto grazie alla mamma, al papà, ad Ale, a Elis, a Bea e a Mario.

Grazie ai miei genitori perché sono per me un esempio, perche’ mi hanno trasmesso stabilita’ e amore, perche’ hanno sempre creduto in me e mi hanno fatto crescere indipendente e capace di prendermi le mie responsabilità. Grazie a Elis e Bea perché mi danno sempre tantissima gioia e mi piace un sacco ascoltare i loro racconti la sera. Grazie ad Ale perché è sempre stato parte di me e anche se adesso vive distante so che c’è sempre.

E grazie a te, Mario, perché hai condiviso questi anni di Università con me giorno per giorno e sai bene cosa hanno significato per me. Grazie perché ci sei e ci sei sempre stato, grazie per quello che sei, grazie per ogni risata, per ogni nuotata, per ogni birra e nachos, per ogni momento meraviglioso passato assieme. E grazie per un libro di altre cose che noi due sappiamo.

Non so cosa mi riservera’ il futuro, ma ho il privilegio di essere felice del mio passato.

“It makes all the difference

if you see the darkness through the light

or the light through the darkness”